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\* \* \* \* \* Welcome to STN International \* \* \* \* \*

NEWS	1		Web Page URLs for STN Seminar Schedule - N. America
NEWS	2	Apr 08	"Ask CAS" for self-help around the clock
NEWS	3	Apr 09	BEILSTEIN: Reload and Implementation of a New Subject Area
NEWS	4	Apr 09	ZDB will be removed from STN
NEWS	5	Apr 19	US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS	6	Apr 22	Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS	7	Apr 22	BIOSIS Gene Names now available in TOXCENTER
NEWS	8	Apr 22	Federal Research in Progress (FEDRIP) now available
NEWS	9	Jun 03	New e-mail delivery for search results now available
NEWS	10	Jun 10	MEDLINE Reload
NEWS	11	Jun 10	PCTFULL has been reloaded
NEWS	12	Jul 02	FOREGE no longer contains STANDARDS file segment
NEWS	13	Jul 22	USAN to be reloaded July 28, 2002; saved answer sets no longer valid
NEWS	14	Jul 29	Enhanced polymer searching in REGISTRY
NEWS	15	Jul 30	NETFIRST to be removed from STN
NEWS	16	Aug 08	CANCERLIT reload
NEWS	17	Aug 08	PHARMAMarketLetter(PHARMAML) - new on STN
NEWS	18	Aug 08	NTIS has been reloaded and enhanced
NEWS	19	Aug 19	Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
NEWS	20	Aug 19	IFIPAT, IFICDB, and IFIUDB have been reloaded
NEWS	21	Aug 19	The MEDLINE file segment of TOXCENTER has been reloaded
NEWS	22	Aug 26	Sequence searching in REGISTRY enhanced
NEWS	23	Sep 03	JAPIO has been reloaded and enhanced
NEWS	24	Sep 16	Experimental properties added to the REGISTRY file
NEWS	25	Sep 16	CA Section Thesaurus available in CAPLUS and CA
NEWS	26	Oct 01	CASREACT Enriched with Reactions from 1907 to 1985
NEWS	27	Oct 21	EVENTLINE has been reloaded
NEWS	28	Oct 24	BEILSTEIN adds new search fields
NEWS	29	Oct 24	Nutraceuticals International (NUTRACEUT) now available on STN
NEWS	30	Oct 25	MEDLINE SDI run of October 8, 2002
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NEWS	38	Dec 30	ISMEC no longer available
NEWS	39	Jan 21	NUTRACEUT offering one free connect hour in February 2003
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NEWS	41	Jan 29	Simultaneous left and right truncation added to COMPENDEX, ENERGY, INSPEC
NEWS	42	Feb 13	CANCERLIT is no longer being updated
NEWS	43	Feb 24	METADEX enhancements
NEWS	44	Feb 24	PCTGEN now available on STN
NEWS	45	Feb 24	TEMA now available on STN

NEWS 46 Feb 26 NTIS now allows simultaneous left and right truncation  
 NEWS 47 Feb 26 PCTFULL now contains images  
 NEWS 48 Mar 04 SDI PACKAGE for monthly delivery of multifile SDI results  
 NEWS 49 Mar 19 APOLLIT offering free connect time in April 2003  
 NEWS 50 Mar 20 EVENTLINE will be removed from STN  
 NEWS 51 Mar 24 PATDPAFULL now available on STN  
 NEWS 52 Mar 24 Additional information for trade-named substances without  
 structures available in REGISTRY  
 NEWS 53 Mar 24 Indexing from 1957 to 1966 added to records in CA/CAPLUS  
  
 NEWS EXPRESS January 6 CURRENT WINDOWS VERSION IS V6.01a,  
 CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),  
 AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002  
 NEWS HOURS STN Operating Hours Plus Help Desk Availability  
 NEWS INTER General Internet Information  
 NEWS LOGIN Welcome Banner and News Items  
 NEWS PHONE Direct Dial and Telecommunication Network Access to STN  
 NEWS WWW CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

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FILE 'HOME' ENTERED AT 15:42:24 ON 28 MAR 2003

=> file medline, uspatful, dgene, embase, wpids, fsta, jicst		
COST IN U.S. DOLLARS	SINCE FILE	TOTAL
	ENTRY	SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'MEDLINE' ENTERED AT 15:42:44 ON 28 MAR 2003

FILE 'USPATFULL' ENTERED AT 15:42:44 ON 28 MAR 2003  
 CA INDEXING COPYRIGHT (C) 2003 AMERICAN CHEMICAL SOCIETY (ACS)

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=> biliprotein  
 BILIPROTEIN IS NOT A RECOGNIZED COMMAND  
 The previous command name entered was not recognized by the system.  
 For a list of commands available to you in the current file, enter

7544

"HELP COMMANDS" at an arrow prompt (=>).

=> s biliprotein

L1 177 BILIPROTEIN

=> s l1 and fusion protein

L2 3 L1 AND FUSION PROTEIN

=> d l2 ti abs ibib tot

L2 ANSWER 1 OF 3 USPATFULL

TI PHYTOFLUORS AS FLUORESCENT LABELS

AB This invention provides new fluorescent molecules useful for detection of target entities. In particular, it relates to fluorescent adducts comprising an apoprotein and a bilin.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2002:37522 USPATFULL

TITLE: PHYTOFLUORS AS FLUORESCENT LABELS

INVENTOR(S): LAGARIAS, JOHN CLARK, DAVIS, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002022239	A1	20020221
APPLICATION INFO.:	US 1999-272809	A1	19990319 (9)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	LAW OFFICES OF JONATHAN ALAN QUINE, PO BOX 458, ALAMEDA, CA, 94501		
NUMBER OF CLAIMS:	32		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	3 Drawing Page(s)		
LINE COUNT:	2727		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 2 OF 3 USPATFULL

TI Recombinant phycobiliprotein and phycobiliprotein linker fusion proteins and uses therefore

AB This invention is directed to the utilization of the developing methods for molecular manipulation of cyanobacteria and red algae (and potentially cryptomonad algae) to express of phycobiliproteins and phycobiliprotein linker fusion proteins and their utilization as phycobiliprotein, phycobilisome and subassembly based reagents. In particular, the present invention relates to a method for a specific binding assay to determine a target moiety which is a member of a specific binding pair, and provides an improvement in the method comprising using a detectable label which is a **fusion protein** containing both a phycobiliprotein domain and another domain corresponding to a first member of a specific binding pair, where the **fusion protein** binds to a second member of the specific binding pair to provide a detectable labeled complex. The domain derived from the first member of the specific binding pair can be directly fused to the phycobiliprotein or phycobiliprotein linker domain or be separated by a spacer that allows correct folding of both domains.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2001:237667 USPATFULL

TITLE: Recombinant phycobiliprotein and phycobiliprotein linker fusion proteins and uses therefore

INVENTOR(S): Allnutt, F.C. Thomas, Port Deposit, MD, United States  
Toole, Colleen Mary, New Winson, MD, United States  
Morseman, John Peter, Columbia, MD, United States

NUMBER	KIND	DATE
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PATENT INFORMATION: US 2001055783 A1 20011227  
APPLICATION INFO.: US 2001-882093 A1 20010618 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-211784P	20000616 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	BROBECK, PHLEGER & HARRISON, LLP, ATTN: INTELLECTUAL PROPERTY DEPARTMENT, 1333 H STREET, N.W. SUITE 800, WASHINGTON, DC, 20005	
NUMBER OF CLAIMS:	46	
EXEMPLARY CLAIM:	1	
LINE COUNT:	1218	
CAS INDEXING IS AVAILABLE FOR THIS PATENT.		

L2 ANSWER 3 OF 3 USPATFULL  
TI Phytofluors as fluorescent labels  
AB This invention provides new fluorescent molecules useful for detection  
of target entities. In particular, it relates to fluorescent adducts  
comprising an apoprotein and a bilin.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
ACCESSION NUMBER: 2000:40859 USPATFULL  
TITLE: Phytofluors as fluorescent labels  
INVENTOR(S): Lagarias, John Clark, Davis, CA, United States  
Murphy, John Thomas, San Francisco, CA, United States  
PATENT ASSIGNEE(S): The Regents of the University of California, Oakland,  
CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6046014		20000404
APPLICATION INFO.:	US 1997-904871		19970801 (8)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-23217P	19960802 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	Granted	
PRIMARY EXAMINER:	Houtteman, Scott W.	
LEGAL REPRESENTATIVE:	Majestic, Parsons, Siebert & Hsue, P.C.	
NUMBER OF CLAIMS:	27	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	37 Drawing Figure(s); 14 Drawing Page(s)	
LINE COUNT:	3666	
CAS INDEXING IS AVAILABLE FOR THIS PATENT.		

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FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, WPIDS, FSTA, JICST-EPLUS'  
ENTERED AT 15:42:44 ON 28 MAR 2003

L1 177 S BILIPROTEIN  
L2 3 S L1 AND FUSION PROTEIN

=> s l1 and holo alpha subunit  
L3 0 L1 AND HOLO ALPHA SUBUNIT

=> s l1 and apo  
L4 1 L1 AND APO

=> d 14 ti abs ibib tot

L4 ANSWER 1 OF 1 USPATFULL  
TI Phytofluors as fluorescent labels  
AB This invention provides new fluorescent molecules useful for detection of target entities. In particular, it relates to fluorescent adducts comprising an apoprotein and a bilin.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2000:40859 USPATFULL  
TITLE: Phytofluors as fluorescent labels  
INVENTOR(S): Lagarias, John Clark, Davis, CA, United States  
Murphy, John Thomas, San Francisco, CA, United States  
PATENT ASSIGNEE(S): The Regents of the University of California, Oakland, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6046014		20000404
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DOCUMENT TYPE:	Utility	
FILE SEGMENT:	Granted	
PRIMARY EXAMINER:	Houtteman, Scott W.	
LEGAL REPRESENTATIVE:	Majestic, Parsons, Siebert & Hsue, P.C.	
NUMBER OF CLAIMS:	27	
EXEMPLARY CLAIM:	1	
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LINE COUNT:	3666	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d his

(FILE 'HOME' ENTERED AT 15:42:24 ON 28 MAR 2003)

FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, WPIDS, FSTA, JICST-EPLUS'  
ENTERED AT 15:42:44 ON 28 MAR 2003

L1 177 S BILIPROTEIN  
L2 3 S L1 AND FUSION PROTEIN  
L3 0 S L1 AND HOLO ALPHA SUBUNIT  
L4 1 S L1 AND APO

=> s l1 and holo

L5 0 L1 AND HOLO

=> s l1 and heme

L6 5 L1 AND HEME

=> d l6 ti abs ibib tot

L6 ANSWER 1 OF 5 MEDLINE  
TI Developmental profile, isolation, and biochemical characterization of a novel lipoglycoheme-carrier protein from the American dog tick, *Dermacentor variabilis* (Acari: Ixodidae) and observations on a similar protein in the soft tick, *Ornithodoros parkeri* (Acari: Argasidae).  
AB A novel lipoglycoheme-carrier protein (CP) in the American dog tick, *Dermacentor variabilis* (Say) has been purified and characterized. CP was purified by native-PAGE from partially fed virgin females. CP has a density of 1.25 g/ml with a molecular weight of 200 K by native-PAGE and

340 K by gel filtration chromatography. CP is comprised of two major subunits, 98 K and 92 K in molecular weight by SDS-PAGE. Separate amino acid composition of the two subunits indicated high contents of As(x), Gl(x) and leucine. However, the N-terminal amino acid sequence of the two subunits was only 13% identical. The lower molecular weight subunit showed 61% identity to artemocyanin (**biliprotein**) in fairy shrimps, 46% identity to minor vitellogenin in chickens and 13% identity to vitellin of the black-legged tick. No similarity match was found for the other subunit. CP is a lipoglycopheme-protein as indicated by selective staining of native-PAGE gel for lipids, carbohydrates and **heme**. Lipid analysis by thin layer chromatography revealed the presence of cholesterol, phospholipids, monoacylglycerides, triacylglycerides and free fatty acids. **Heme** associated with purified CP demonstrated a lambda(max) of 397.5 nm while the lambda(max) of crude hemolymph plasma was 402.5 nm. The presence of CP in whole body homogenates of eggs, unfed and fed larvae and fed nymphs as well as in the plasma of unfed and fed adults including vitellogenic females was demonstrated by native-PAGE. Although a protein of analogous size was not found in the soft tick, *Ornithodoros parkeri* Cooley, a high molecular weight protein (500 K) is the predominant plasma protein in both unfed and fed male and female adults of that species as determined by native-PAGE. Also, CP appears to function as a **biliprotein** which sequesters **heme**.

ACCESSION NUMBER: 2001236262 MEDLINE  
DOCUMENT NUMBER: 21124779 PubMed ID: 11222939  
TITLE: Developmental profile, isolation, and biochemical characterization of a novel lipoglycopheme-carrier protein from the American dog tick, *Dermacentor variabilis* (Acari: Ixodidae) and observations on a similar protein in the soft tick, *Ornithodoros parkeri* (Acari: Argasidae).  
AUTHOR: Gudderra N P; Neese P A; Sonenshine D E; Apperson C S; Roe R M  
CORPORATE SOURCE: Department of Entomology, North Carolina State University, Raleigh, NC 27695-7647, USA.  
CONTRACT NUMBER: 1 RO1 AI 36257 (NIAID)  
SOURCE: INSECT BIOCHEMISTRY AND MOLECULAR BIOLOGY, (2001 Mar 15) 31 (4-5) 299-311.  
Journal code: 9207282. ISSN: 0965-1748.  
PUB. COUNTRY: England: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200105  
ENTRY DATE: Entered STN: 20010517  
Last Updated on STN: 20010517  
Entered Medline: 20010503

L6 ANSWER 2 OF 5 MEDLINE

TI Coupled oxidation of **heme** covalently attached to cytochrome b562 yields a novel **biliprotein**.

AB A variant of *Escherichia coli* cytochrome b(562) with covalently attached **heme** can be converted to a biliverdin-containing protein in two distinct stages by coupled oxidation and acid hydrolysis. The first stage of coupled oxidation yields a stable verdoheme-containing protein. This verdoheme protein is unusual in three respects. First, the verdoheme group is covalently bound to the protein through a c-type thioether linkage. Second, the oxidation stops at the verdoheme stage, and finally, this is the first report of verdoheme generated from a **heme** protein with exclusive methionine ligation to the **heme** iron. In addition, the oxidation process does not require denaturation of the protein. The product has been characterized by optical spectroscopy, ESI mass spectrometry, and (1)H NMR. The NMR data show that the predominant product is the result of oxidation at the alpha-meso carbon. A collective evaluation of data on the topic suggests that the electronic structure of the **heme**, not protein steric effects, is the main factor in

controlling the regiospecificity of the oxidation site. In the second stage of conversion to a **biliprotein**, we demonstrate that the verdoheme ring can be opened by treatment with aqueous formic acid to give alpha-biliverdin covalently attached to the folded protein. This product, a protein-bound linear tetrapyrrole as characterized by optical spectroscopy and mass spectrometry, is an example of a phycobilin chromophore that has not been observed previously.

ACCESSION NUMBER: 2000074535 MEDLINE  
DOCUMENT NUMBER: 20074535 PubMed ID: 10606518  
TITLE: Coupled oxidation of **heme** covalently attached to cytochrome b562 yields a novel **biliprotein**.  
AUTHOR: Rice J K; Fearnley I M; Barker P D  
CORPORATE SOURCE: Naval Research Laboratory, Washington, D.C. 20375-5342, USA.  
SOURCE: BIOCHEMISTRY, (1999 Dec 21) 38 (51) 16847-56.  
Journal code: 0370623. ISSN: 0006-2960.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200001  
ENTRY DATE: Entered STN: 20000131  
Last Updated on STN: 20000131  
Entered Medline: 20000119

L6 ANSWER 3 OF 5 MEDLINE  
TI The molecular structure of insecticyanin from the tobacco hornworm *Manduca sexta* L. at 2.6 A resolution.  
AB Insecticyanin, a blue **biliprotein** isolated from the tobacco hornworm *Manduca sexta* L., is involved in insect camouflage. Its three-dimensional structure has now been solved to 2.6 A resolution using the techniques of multiple isomorphous replacement, non-crystallographic symmetry averaging about a local 2-fold rotation axis and solvent flattening. All 189 amino acids have been fitted to the electron density map. The map clearly shows that insecticyanin is a tetramer with one of its molecular 2-fold axes coincident to a crystallographic dyad. The individual subunits have overall dimensions of 44 A X 37 A X 40 A and consist primarily of an eight-stranded anti-parallel beta-barrel flanked on one side by a 4.5-turn alpha-helix. Interestingly the overall three-dimensional fold of the insecticyanin subunit shows remarkable similarity to the structural motifs of bovine beta-lactoglobulin and the human serum retinol-binding protein. The electron density attributable to the chromophore is unambiguous and shows that it is indeed the gamma-isomer of biliverdin. The biliverdin lies towards the open end of the beta-barrel with its two propionate side chains pointing towards the solvent and it adopts a rather folded conformation, much like a **heme**.

ACCESSION NUMBER: 87275848 MEDLINE  
DOCUMENT NUMBER: 87275848 PubMed ID: 3608987  
TITLE: The molecular structure of insecticyanin from the tobacco hornworm *Manduca sexta* L. at 2.6 A resolution.  
AUTHOR: Holden H M; Rypniewski W R; Law J H; Rayment I  
CONTRACT NUMBER: AM GM 351865 (NIADDK)  
BRSG 829023 (DRS)  
GM 29238 (NIGMS)  
SOURCE: EMBO JOURNAL, (1987 Jun) 6 (6) 1565-70.  
Journal code: 8208664. ISSN: 0261-4189.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198709  
ENTRY DATE: Entered STN: 19900305  
Last Updated on STN: 19970203

L6 ANSWER 4 OF 5 USPATFULL

TI Reduction of oxyradical damage in biomedical applications

AB The biliproteins delta-bilirubin and delta-bilipeptide are useful as a cytoprotective antioxidants. Delta-bilipeptide as the term is used herein is a truncated form of delta-bilirubin in which an albumin analogue of 10-200 amino acid residues replaces the albumin portion of delta-bilirubin. Patient-administrable compositions for addition to a patient's blood to minimize oxyradical damage caused by ischemia-reperfusion injury that may result in various surgical procedures, and comprising delta-bilirubin or delta-bilipeptide, are described.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 91:73347 USPATFULL

TITLE: Reduction of oxyradical damage in biomedical applications

INVENTOR(S): Wu, Tai-Wing, Toronto, Canada

PATENT ASSIGNEE(S): Nagase Co., Ltd., Osaka, Japan (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5047395		19910910
APPLICATION INFO.:	US 1990-554197		19900717 (7)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Waddell, Frederick E.		
ASSISTANT EXAMINER:	Wilson, Terry		
LEGAL REPRESENTATIVE:	Wyatt, Gerber, Burke and Badie		
NUMBER OF CLAIMS:	20		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	1 Drawing Figure(s); 1 Drawing Page(s)		
LINE COUNT:	430		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 5 OF 5 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI Coupled oxidation of **heme** covalently attached to cytochrome b562 yields a novel **biliprotein**.

AB A variant of Escherichia coli cytochrome b562 with covalently attached **heme** can be converted to a biliverdin-containing protein in two distinct stages by coupled oxidation and acid hydrolysis. The first stage of coupled oxidation yields a stable verdoheme-containing protein. This verdoheme protein is unusual in three respects. First, the verdoheme group is covalently bound to the protein through a c-type thioether linkage. Second, the oxidation stops at the verdoheme stage, and finally, this is the first report of verdoheme generated from a **heme** protein with exclusive methionine ligation to the **heme** iron. In addition, the oxidation process does not require denaturation of the protein. The product has been characterized by optical spectroscopy, ESI mass spectrometry, and <sup>1</sup>H NMR. The NMR data show that the predominant product is the result of oxidation at the .alpha.-meso carbon. A collective evaluation of data on the topic suggests that the electronic structure of the **heme**, not protein steric effects, is the main factor in controlling the regiospecificity of the oxidation site. In the second stage of conversion to a **biliprotein**, we demonstrate that the verdoheme ring can be opened by treatment with aqueous formic acid to give .alpha.-biliverdin covalently attached to the folded protein. This product, a protein-bound linear tetrapyrrole as characterized by optical spectroscopy and mass spectrometry, is an example of a phycobilin chromophore that has not been observed previously.

ACCESSION NUMBER: 2000009879 EMBASE

TITLE: Coupled oxidation of **heme** covalently attached to cytochrome b562 yields a novel **biliprotein**.

AUTHOR: Rice J.K.; Fearnley I.M.; Barker P.D.  
CORPORATE SOURCE: P.D. Barker, Centre for Protein Engineering, MRC Centre,  
Hills Road, Cambridge CB2 2QH, United Kingdom.  
pxb@mrc-lmb.cam.ac.uk  
SOURCE: Biochemistry, (21 Dec 1999) 38/51 (16847-16856).  
Refs: 36  
ISSN: 0006-2960 CODEN: BICHAW  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

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=> s phycoerythrocyanin

L1 143 PHYCOERYTHROCYANIN

=> s holo alpha subunit

L2 9 HOLO ALPHA SUBUNIT

=> d l2 and l1

L1 IS NOT VALID HERE

For an explanation, enter "HELP DISPLAY".

=> s l2 and l1

L3 3 L2 AND L1

=> d l2 ti abs ibib tot

L2 ANSWER 1 OF 9 MEDLINE

TI Biosynthesis of the cyanobacterial light-harvesting polypeptide phycoerythrocyanin **holo-alpha subunit** in a heterologous host.

AB The entire pathway for the biosynthesis of the phycobiliviolin-bearing His-tagged **holo-alpha subunit** of the cyanobacterial photosynthetic accessory protein phycoerythrocyanin was reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes required for the conversion of heme to 3Z-phycocyanobilin, a precursor of phycobiliviolin (namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase), were expressed from a plasmid under the control of the hybrid trp-lac (trc) promoter. Genes for the apo-phycoerythrocyanin alpha subunit (pecA) and the heterodimeric lyase/isomerase (pecE and pecF), which catalyzes both the covalent attachment of phycocyanobilin and its concurrent isomerization to phycobiliviolin, were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used endogenous heme to produce holo-PecA with absorbance and fluorescence properties similar to those of the same protein produced in cyanobacteria. About two-thirds of the apo-PecA was converted to holo-PecA. No significant bilin addition took place in a similarly engineered E. coli strain that lacks pecE and pecF. By using immobilized metal affinity chromatography, both apo-PecA and holo-PecA were isolated as ternary complexes with PecE and PecF. The identities of all three components in the ternary complexes were established unambiguously by protein and tryptic peptide analyses performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

ACCESSION NUMBER: 2002415572 MEDLINE

DOCUMENT NUMBER: 22159919 PubMed ID: 12169589

TITLE: Biosynthesis of the cyanobacterial light-harvesting polypeptide phycoerythrocyanin **holo-alpha subunit** in a heterologous host.

AUTHOR: Tooley Aaron J; Glazer Alexander N

CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200, USA.

SOURCE: JOURNAL OF BACTERIOLOGY, (2002 Sep) 184 (17) 4666-71.  
Journal code: 2985120R. ISSN: 0021-9193.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200208

ENTRY DATE: Entered STN: 20020810

Last Updated on STN: 20020830

Entered Medline: 20020829

L2 ANSWER 2 OF 9 MEDLINE

TI Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo-alpha subunit** in a heterologous host.

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein

*Valid date*

subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phytyocyanobilin, namely, heme oxygenase 1 and 3Z-phytyocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid *trp-lac* (*trc*) promoter. Genes for the apoprotein (C-phytyocyanin alpha subunit; *cpcA*) and the heterodimeric lyase (*cpcE* and *cpcF*) that catalyzes chromophore attachment were expressed from the *trc* promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks *cpcE* and *cpcF*. This approach should permit incisive analysis of many remaining questions in phytyobiliprotein biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins *in situ* for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001504133 MEDLINE  
DOCUMENT NUMBER: 21438034 PubMed ID: 11553806  
TITLE: Biosynthesis of a fluorescent cyanobacterial C-phytyocyanin **holo-alpha subunit** in a heterologous host.  
AUTHOR: Tooley A J; Cai Y A; Glazer A N  
CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, 142 LSA no. 3200, Berkeley, CA 94720-3200, USA.  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Sep 11) 98 (19) 10560-5. Journal code: 7505876. ISSN: 0027-8424.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200111  
ENTRY DATE: Entered STN: 20010913  
Last Updated on STN: 20011105  
Entered Medline: 20011101

L2 ANSWER 3 OF 9 MEDLINE

TI Phytyocyanin alpha-subunit phytyocyanobilin lyase.

AB Phytyobiliproteins, unlike other light-harvesting proteins involved in photosynthesis, bear covalently attached chromophores. The bilin chromophores are attached through thioether bonds to cysteine residues. The cyanobacterium *Synechococcus* sp. PCC 7002 has eight distinct bilin attachment sites on seven polypeptides, all of which carry the same chromophore, phytyocyanobilin. When two genes in the phytyocyanin operon of this organism, *cpcE* and *cpcF*, are inactivated by insertion, together or separately, the surprising result is elimination of correct bilin attachment at only one site, that on the alpha subunit of phytyocyanin. We have overproduced CpcE and CpcF in *Escherichia coli*. *In vitro*, these proteins catalyze the attachment of phytyocyanobilin to the alpha subunit of apophytyocyanin at the appropriate site, alpha-Cys-84, to form the correct adduct. CpcE and CpcF also efficiently catalyze the reverse reaction, in which the bilin from **holo-alpha subunit** is transferred either to the apo-alpha subunit of the same C-phytyocyanin or to the apo-alpha subunit of a heterologous C-phytyocyanin. The forward and reverse reactions each require both CpcE and CpcF and are specific for the alpha-Cys-84 position. Phytyocyanobilin is the immediate precursor of the protein-bound bilin.

ACCESSION NUMBER: 92357762 MEDLINE  
DOCUMENT NUMBER: 92357762 PubMed ID: 1495995  
TITLE: Phytyocyanin alpha-subunit phytyocyanobilin lyase.  
AUTHOR: Fairchild C D; Zhao J; Zhou J; Colson S E; Bryant D A; Glazer A N

CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, Berkeley 94720.  
CONTRACT NUMBER: GM28994 (NIGMS)  
GM31625 (NIGMS)  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 Aug 1) 89 (15) 7017-21. Journal code: 7505876. ISSN: 0027-8424.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199209  
ENTRY DATE: Entered STN: 19920925  
Last Updated on STN: 19970203  
Entered Medline: 19920904

L2 ANSWER 4 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

TI Biosynthesis of the cyanobacterial light-harvesting polypeptide phycoerythrocyanin **holo-alpha subunit** in a heterologous host.

AB The entire pathway for the biosynthesis of the phycobiliviolin-bearing His-tagged **holo-alpha subunit** of the cyanobacterial photosynthetic accessory protein phycoerythrocyanin was reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes required for the conversion of heme to 3Z-phycoerythrocyanin, a precursor of phycobiliviolin (namely, heme oxygenase 1 and 3Z-phycoerythrocyanin:ferredoxin oxidoreductase), were expressed from a plasmid under the control of the hybrid trp-lac (trc) promoter. Genes for the apo-phycoerythrocyanin alpha subunit (pecA) and the heterodimeric lyase/isomerase (pecE and pecF), which catalyzes both the covalent attachment of phycoerythrocyanin and its concurrent isomerization to phycobiliviolin, were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used endogenous heme to produce holo-PecA with absorbance and fluorescence properties similar to those of the same protein produced in cyanobacteria. About two-thirds of the apo-PecA was converted to holo-PecA. No significant bilin addition took place in a similarly engineered E. coli strain that lacks pecE and pecF. By using immobilized metal affinity chromatography, both apo-PecA and holo-PecA were isolated as ternary complexes with PecE and PecF. The identities of all three components in the ternary complexes were established unambiguously by protein and tryptic peptide analyses performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

ACCESSION NUMBER: 2002:464629 BIOSIS

DOCUMENT NUMBER: PREV200200464629

TITLE: Biosynthesis of the cyanobacterial light-harvesting polypeptide phycoerythrocyanin **holo-alpha subunit** in a heterologous host.

AUTHOR(S): Tooley, Aaron J.; Glazer, Alexander N. (1)

CORPORATE SOURCE: (1) Natural Reserve System, University of California, 1111 Franklin Street, 6th Floor, Oakland, CA, 94607-5200: glazer@uclink4.berkeley.edu USA

SOURCE: Journal of Bacteriology, (September, 2002) Vol. 184, No. 17, pp. 4666-4671. <http://intl-jb.asm.org/>. print. ISSN: 0021-9193.

DOCUMENT TYPE: Article

LANGUAGE: English

L2 ANSWER 5 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

TI Biosynthesis of a fluorescent cyanobacterial C-phycoerythrocyanin **holo-alpha subunit** in a heterologous host.

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (Synechocystis sp. PCC6803) was reconstituted in Escherichia coli. Cyanobacterial genes encoding

enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phycocyanin alpha subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin addition took place in a similarly engineered E. coli strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in phycobiliprotein biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001:482056 BIOSIS  
DOCUMENT NUMBER: PREV200100482056  
TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo-**alpha** subunit in a heterologous host.  
AUTHOR(S): Tooley, Aaron J.; Cai, Yuping A.; Glazer, Alexander N. (1)  
CORPORATE SOURCE: (1) Natural Reserve System, University of California System, 1111 Franklin Street, 6th Floor, Oakland, CA, 94607-5200: alexander.glazer@ucop.edu USA  
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (September 11, 2001) Vol. 98, No. 19, pp. 10560-10565. print.  
ISSN: 0027-8424.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
SUMMARY LANGUAGE: English

L2 ANSWER 6 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

TI PHYCOCYANIN ALPHA-SUBUNIT PHYCOCYANOBILIN LYASE.

AB Phycobiliproteins, unlike other light-harvesting proteins involved in photosynthesis, bear covalently attached chromophores. The bilin chromophores are attached through thioether bonds to cysteine residues. The cyanobacterium Synechococcus sp. PCC 7002 has eight distinct bilin attachment sites on seven polypeptides, all of which carry the same chromophore, phycocyanobilin. When two genes in the phycocyanin operon of this organisms, cpcE and cpcF, are inactivated by insertion, together or separately, the suprising result is elimination of correct bilin attachment at only one site, that on the .alpha. subunit of phycocyanin. We have overproduced CpcE and CpcF in Escherichia coli. In vitro, these proteins catalyze the attachment of phycocyanobilin to the .alpha. subunit of apophycocyanin at the appropriate site, .alpha. Cys-84, to form the correct adduct. CpcE and CpcF also efficiently catalyze the reverse reaction, in which the bilin from holo-.alpha. subunit is transferred either to the apo-.alpha. subunit of the same C-phycocyanin or to the apo-.alpha. subunit of a heterologous C-phycocyanin. The forward and reverse reactions each require both CpcE and CpcF and are specific for the .alpha.-Cys-84 position. Phycocyanobilin is the immediate precursor of the protein-bound bilin.

ACCESSION NUMBER: 1992:506225 BIOSIS  
DOCUMENT NUMBER: BA94:124750  
TITLE: PHYCOCYANIN ALPHA-SUBUNIT PHYCOCYANOBILIN LYASE.  
AUTHOR(S): FAIRCHILD C D; ZHAO J; ZHOU J; COLSON S E; BRYANT D A; GLAZER A N  
CORPORATE SOURCE: MCB: STANLEY/DONNER ASU, 229 STANLEY HALL, UNIV. CALIF., BERKELEY, CALIF. 94720.  
SOURCE: PROC NATL ACAD SCI U S A, (1992) 89 (15), 7017-7021.  
CODEN: PNASA6. ISSN: 0027-8424.  
FILE SEGMENT: BA; OLD

LANGUAGE: English

L2 ANSWER 7 OF 9 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI Biosynthesis of the cyanobacterial light-harvesting polypeptide phycoerythrocyanin **holo-.alpha. subunit** in a heterologous host.

AB The entire pathway for the biosynthesis of the phycobiliviolin-bearing His-tagged **holo-.alpha. subunit** of the cyanobacterial photosynthetic accessory protein phycoerythrocyanin was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to 3Z-phycoerythrobilin, a precursor of phycobiliviolin (namely, heme oxygenase 1 and 3Z-phycoerythrobilin:ferredoxin oxidoreductase), were expressed from a plasmid under the control of the hybrid trp-lac (trc) promoter. Genes for the apo-phycoerythrocyanin **.alpha. subunit** (pecA) and the heterodimeric lyase/isomerase (pecE and pecF), which catalyzes both the covalent attachment of phycoerythrobilin and its concurrent isomerization to phycobiliviolin, were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used endogenous heme to produce **holo-PecA** with absorbance and fluorescence properties similar to those of the same protein produced in cyanobacteria. About two-thirds of the apo-PecA was converted to **holo-PecA**. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks pecE and pecF. By using immobilized metal affinity chromatography, both apo-PecA and **holo-PecA** were isolated as ternary complexes with PecE and PecF. The identities of all three components in the ternary complexes were established unambiguously by protein and tryptic peptide analyses performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

ACCESSION NUMBER: 2002295599 EMBASE

TITLE: Biosynthesis of the cyanobacterial light-harvesting polypeptide phycoerythrocyanin **holo-.alpha. subunit** in a heterologous host.

AUTHOR: Tooley A.J.; Glazer A.N.

CORPORATE SOURCE: A.N. Glazer, Natural Reserve System, University of California, 1111 Franklin Street, Oakland, CA 94607-5200, United States. glazer@uclink4.berkeley.edu

SOURCE: Journal of Bacteriology, (2002) 184/17 (4666-4671).

Refs: 22

ISSN: 0021-9193 CODEN: JOBAAY

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

L2 ANSWER 8 OF 9 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI Biosynthesis of a fluorescent cyanobacterial C-phycoerythrin **holo-.alpha. subunit** in a heterologous host.

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycoerythrobilin, namely, heme oxygenase 1 and 3Z-phycoerythrobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phycoerythrin **.alpha. subunit**; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce **holo-CpcA** with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to **holo-CpcA**. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks cpcE and

cpcF. This approach should permit incisive analysis of many remaining questions in phycobiliprotein biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001329835 EMBASE  
TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin  
          **holo-.alpha. subunit** in a  
          heterologous host.  
AUTHOR: Tooley A.J.; Cai Y.A.; Glazer A.N.  
CORPORATE SOURCE: A.N. Glazer, Natural Reserve System, University of  
                  California System, 1111 Franklin Street, Oakland, CA  
                  94607-5200, United States. alexander.glazer@ucop.edu  
SOURCE: Proceedings of the National Academy of Sciences of the  
          United States of America, (11 Sep 2001) 98/19  
          (10560-10565).  
          Refs: 30  
          ISSN: 0027-8424 CODEN: PNASA6  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

L2 ANSWER 9 OF 9 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
TI Phycocyanin .alpha.-subunit phycocyanobilin lyase.  
AB Phycobiliproteins, unlike other light-harvesting proteins involved in  
photosynthesis, bear covalently attached chromophores. The bilin  
chromophores are attached through thioether bonds to cysteine residues.  
The cyanobacterium *Synechococcus* sp. PCC 7002 has eight distinct bilin  
attachment sites on seven polypeptides, all of which carry the same  
chromophore, phycocyanobilin. When two genes in the phycocyanin operon of  
this organism, *cpcE* and *cpcF*, are inactivated by insertion, together or  
separately, the surprising result is elimination of correct bilin  
attachment at only one site, that on the .alpha. subunit of phycocyanin.  
We have overproduced *CpcE* and *CpcF* in *Escherichia coli*. In vitro, these  
proteins catalyze the attachment of phycocyanobilin to the .alpha. subunit  
of apophycocyanin at the appropriate site, .alpha.-Cys-84, to form the  
correct adduct. *CpcE* and *CpcF* also efficiently catalyze the reverse  
reaction, in which the bilin from **holo-.alpha.**  
**subunit** is transferred either to the apo-.alpha. subunit of the  
same C-phycocyanin or to the apo-.alpha. subunit of a heterologous  
C-phycocyanin. The forward and reverse reactions each require both *CpcE*  
and *CpcF* and are specific for the .alpha.-Cys-84 position. Phycocyanobilin  
is the immediate precursor of the protein-bound bilin.

ACCESSION NUMBER: 92240711 EMBASE  
DOCUMENT NUMBER: 1992240711  
TITLE: Phycocyanin .alpha.-subunit phycocyanobilin lyase.  
AUTHOR: Fairchild C.D.; Zhao J.; Zhou J.; Colson S.E.; Bryant D.A.;  
          Glazer A.N.  
CORPORATE SOURCE: MCB: Stanley/Donner ASU, 229 Stanley Hall, University of  
                  California, Berkeley, CA 94720, United States  
SOURCE: Proceedings of the National Academy of Sciences of the  
          United States of America, (1992) 89/15 (7017-7021).  
          ISSN: 0027-8424 CODEN: PNASA6  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 029 Clinical Biochemistry  
LANGUAGE: English  
SUMMARY LANGUAGE: English

=> d his

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FILE 'MEDLINE, BIOSIS, EMBASE, DGENE, WPIDS, FSTA, JICST-EPLUS, JAPIO'  
ENTERED AT 15:17:25 ON 28 MAR 2003

L1 143 S PHYCOERYTHROCYANIN  
L2 9 S HOLO ALPHA SUBUNIT  
L3 3 S L2 AND L1

=> d l3 ti abs ibib tot

L3 ANSWER 1 OF 3 MEDLINE

TI Biosynthesis of the cyanobacterial light-harvesting polypeptide  
**phycoerythrocyanin holo-alpha subunit**  
in a heterologous host.

AB The entire pathway for the biosynthesis of the phycobiliviolin-bearing His-tagged **holo-alpha subunit** of the cyanobacterial photosynthetic accessory protein **phycoerythrocyanin** was reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes required for the conversion of heme to 3Z-phycocyanobilin, a precursor of phycobiliviolin (namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase), were expressed from a plasmid under the control of the hybrid trp-lac (trc) promoter. Genes for the apo-**phycoerythrocyanin** alpha subunit (pecA) and the heterodimeric lyase/isomerase (pecE and pecF), which catalyzes both the covalent attachment of phycocyanobilin and its concurrent isomerization to phycobiliviolin, were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used endogenous heme to produce holo-PecA with absorbance and fluorescence properties similar to those of the same protein produced in cyanobacteria. About two-thirds of the apo-PecA was converted to holo-PecA. No significant bilin addition took place in a similarly engineered E. coli strain that lacks pecE and pecF. By using immobilized metal affinity chromatography, both apo-PecA and holo-PecA were isolated as ternary complexes with PecE and PecF. The identities of all three components in the ternary complexes were established unambiguously by protein and tryptic peptide analyses performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

ACCESSION NUMBER: 2002415572 MEDLINE  
DOCUMENT NUMBER: 22159919 PubMed ID: 12169589  
TITLE: Biosynthesis of the cyanobacterial light-harvesting polypeptide **phycoerythrocyanin holo-alpha subunit** in a heterologous host.  
AUTHOR: Tooley Aaron J; Glazer Alexander N  
CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200, USA.  
SOURCE: JOURNAL OF BACTERIOLOGY, (2002 Sep) 184 (17) 4666-71.  
Journal code: 2985120R. ISSN: 0021-9193.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200208  
ENTRY DATE: Entered STN: 20020810  
Last Updated on STN: 20020830  
Entered Medline: 20020829

L3 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

TI Biosynthesis of the cyanobacterial light-harvesting polypeptide  
**phycoerythrocyanin holo-alpha subunit**  
in a heterologous host.

AB The entire pathway for the biosynthesis of the phycobiliviolin-bearing His-tagged **holo-alpha subunit** of the cyanobacterial photosynthetic accessory protein **phycoerythrocyanin** was reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes required for the conversion of heme to 3Z-phycocyanobilin, a

precursor of phycobiliviolin (namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase), were expressed from a plasmid under the control of the hybrid trp-lac (trc) promoter. Genes for the apo-**phycoerythrocyanin** alpha subunit (pecA) and the heterodimeric lyase/isomerase (pecE and pecF), which catalyzes both the covalent attachment of phycocyanobilin and its concurrent isomerization to phycobiliviolin, were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used endogenous heme to produce holo-PecA with absorbance and fluorescence properties similar to those of the same protein produced in cyanobacteria. About two-thirds of the apo-PecA was converted to holo-PecA. No significant bilin addition took place in a similarly engineered E. coli strain that lacks pecE and pecF. By using immobilized metal affinity chromatography, both apo-PecA and holo-PecA were isolated as ternary complexes with PecE and PecF. The identities of all three components in the ternary complexes were established unambiguously by protein and tryptic peptide analyses performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

ACCESSION NUMBER: 2002:464629 BIOSIS  
DOCUMENT NUMBER: PREV200200464629  
TITLE: Biosynthesis of the cyanobacterial light-harvesting polypeptide **phycoerythrocyanin holo-alpha subunit** in a heterologous host.  
AUTHOR(S): Tooley, Aaron J.; Glazer, Alexander N. (1)  
CORPORATE SOURCE: (1) Natural Reserve System, University of California, 1111 Franklin Street, 6th Floor, Oakland, CA, 94607-5200: glazer@uclink4.berkeley.edu USA  
SOURCE: Journal of Bacteriology, (September, 2002) Vol. 184, No. 17, pp. 4666-4671. <http://intl-jb.asm.org/>. print. ISSN: 0021-9193.  
DOCUMENT TYPE: Article  
LANGUAGE: English

L3 ANSWER 3 OF 3 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI Biosynthesis of the cyanobacterial light-harvesting polypeptide **phycoerythrocyanin holo-.alpha. subunit** in a heterologous host.

AB The entire pathway for the biosynthesis of the phycobiliviolin-bearing His-tagged **holo-.alpha. subunit** of the cyanobacterial photosynthetic accessory protein **phycoerythrocyanin** was reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes required for the conversion of heme to 3Z-phycocyanobilin, a precursor of phycobiliviolin (namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase), were expressed from a plasmid under the control of the hybrid trp-lac (trc) promoter. Genes for the apo-**phycoerythrocyanin .alpha. subunit** (pecA) and the heterodimeric lyase/isomerase (pecE and pecF), which catalyzes both the covalent attachment of phycocyanobilin and its concurrent isomerization to phycobiliviolin, were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used endogenous heme to produce holo-PecA with absorbance and fluorescence properties similar to those of the same protein produced in cyanobacteria. About two-thirds of the apo-PecA was converted to holo-PecA. No significant bilin addition took place in a similarly engineered E. coli strain that lacks pecE and pecF. By using immobilized metal affinity chromatography, both apo-PecA and holo-PecA were isolated as ternary complexes with PecE and PecF. The identities of all three components in the ternary complexes were established unambiguously by protein and tryptic peptide analyses performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

ACCESSION NUMBER: 2002295599 EMBASE  
TITLE: Biosynthesis of the cyanobacterial light-harvesting polypeptide **phycoerythrocyanin holo-.alpha. subunit** in a heterologous host.

AUTHOR: Tooley A.J.; Glazer A.N.  
 CORPORATE SOURCE: A.N. Glazer, Natural Reserve System, University of California, 1111 Franklin Street, Oakland, CA 94607-5200, United States. glazer@uclink4.berkeley.edu  
 SOURCE: Journal of Bacteriology, (2002) 184/17 (4666-4671).  
 Refs: 22  
 ISSN: 0021-9193 CODEN: JOBAAY  
 COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article  
 FILE SEGMENT: 004 Microbiology  
 LANGUAGE: English  
 SUMMARY LANGUAGE: English

=> d his

(FILE 'HOME' ENTERED AT 15:16:17 ON 28 MAR 2003)

FILE 'MEDLINE, BIOSIS, EMBASE, DGENE, WPIDS, FSTA, JICST-EPLUS, JAPIO'  
 ENTERED AT 15:17:25 ON 28 MAR 2003

L1 143 S PHYCOERYTHROCYANIN  
 L2 9 S HOLO ALPHA SUBUNIT  
 L3 3 S L2 AND L1

=> s phycobiliviolin  
 L4 21 PHYCOBILIVIOLIN

=> s phycobiliprotein  
 L5 724 PHYCOBILIPROTEIN

=> s l1 and l5  
 L6 39 L1 AND L5

=> s l6 and l2  
 L7 1 L6 AND L2

=> d l7 ti abs ibib tot

L7 ANSWER 1 OF 1 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
 TI Biosynthesis of the cyanobacterial light-harvesting polypeptide **phycoerythrocyanin holo-.alpha. subunit** in a heterologous host.  
 AB The entire pathway for the biosynthesis of the phycobiliviolin-bearing His-tagged **holo-.alpha. subunit** of the cyanobacterial photosynthetic accessory protein **phycoerythrocyanin** was reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes required for the conversion of heme to 3Z-phycocyanobilin, a precursor of phycobiliviolin (namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase), were expressed from a plasmid under the control of the hybrid trp-lac (trc) promoter. Genes for the apo-**phycoerythrocyanin .alpha. subunit** (pecA) and the heterodimeric lyase/isomerase (pecE and pecF), which catalyzes both the covalent attachment of phycocyanobilin and its concurrent isomerization to phycobiliviolin, were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used endogenous heme to produce holo-PecA with absorbance and fluorescence properties similar to those of the same protein produced in cyanobacteria. About two-thirds of the apo-PecA was converted to holo-PecA. No significant bilin addition took place in a similarly engineered E. coli strain that lacks pecE and pecF. By using immobilized metal affinity chromatography, both apo-PecA and holo-PecA were isolated as ternary complexes with PecE and PecF. The identities of all three components in the ternary complexes were established unambiguously by protein and tryptic peptide analyses performed by matrix-assisted laser desorption ionization-time of flight

mass spectrometry.

ACCESSION NUMBER: 2002295599 EMBASE

TITLE: Biosynthesis of the cyanobacterial light-harvesting polypeptide **phycoerythrocyanin** holo-. **alpha. subunit** in a heterologous host.

AUTHOR: Tooley A.J.; Glazer A.N.

CORPORATE SOURCE: A.N. Glazer, Natural Reserve System, University of California, 1111 Franklin Street, Oakland, CA 94607-5200, United States. glazer@uclink4.berkeley.edu

SOURCE: Journal of Bacteriology, (2002) 184/17 (4666-4671). Refs: 22  
ISSN: 0021-9193 CODEN: JOBAAY

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

=> d his

(FILE 'HOME' ENTERED AT 15:16:17 ON 28 MAR 2003)

FILE 'MEDLINE, BIOSIS, EMBASE, DGENE, WPIDS, FSTA, JICST-EPLUS, JAPIO'  
ENTERED AT 15:17:25 ON 28 MAR 2003

L1 143 S PHYCOERYTHROCYANIN

L2 9 S HOLO ALPHA SUBUNIT

L3 3 S L2 AND L1

L4 21 S PHYCOBILIVIOLIN

L5 724 S PHYCOBILIPROTEIN

L6 39 S L1 AND L5

L7 1 S L6 AND L2

=> d l6 ti abs ibib 1-15

L6 ANSWER 1 OF 39 MEDLINE

TI Novel activity of a **phycobiliprotein** lyase: both the attachment of phycocyanobilin and the isomerization to phycoviolobilin are catalyzed by the proteins PecE and PecF encoded by the **phycoerythrocyanin** operon.

AB The structure of phycoviolobilin, the photoactive chromophore of alpha-**phycoerythrocyanin**, is incompatible with a chromophore ligation to the apoprotein via SH-addition (cysteine) to a Delta3, 3(1)-double bond of the phycobilin. The two putative **phycoerythrocyanin** lyase genes of *Mastigocladus laminosus*, pecE and pecF, were overexpressed in *Escherichia coli*. Their action has been studied on the addition reaction of phycocyanobilin to apo-alpha-**phycoerythrocyanin** (PecA). In the absence of the components of alpha-PEC-phycoviolobilin lyase PecE and PecF, or in the presence of only one of them, phycocyanobilin binds covalently to PecA forming a fluorescent chromoprotein with a red-shifted absorption (lambda(max)=641 nm) and low photoactivity (<10%). In the presence of both PecE and PecF, a chromoprotein forms which by its absorption (lambda(max)=565 nm) and high photoreversible photochromism (100% type I) has been identified as integral alpha-**phycoerythrocyanin**. We conclude that PecE and PecF jointly catalyze not only the addition of phycocyanobilin to PecA, but also its isomerization to the native phycoviolobilin chromophore.

ACCESSION NUMBER: 2000175401 MEDLINE

DOCUMENT NUMBER: 20175401 PubMed ID: 10708746

TITLE: Novel activity of a **phycobiliprotein** lyase: both the attachment of phycocyanobilin and the isomerization to phycoviolobilin are catalyzed by the proteins PecE and PecF encoded by the **phycoerythrocyanin** operon.

AUTHOR: Zhao K H; Deng M G; Zheng M; Zhou M; Parbel A; Storf M;

CORPORATE SOURCE: Meyer M; Strohmam B; Scheer H  
College of Life Sciences, Wuhan University, Wuhan, PR  
China.. khzhao@public.wh.hb.cn  
SOURCE: FEBS LETTERS, (2000 Mar 3) 469 (1) 9-13.  
Journal code: 0155157. ISSN: 0014-5793.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200004  
ENTRY DATE: Entered STN: 20000421  
Last Updated on STN: 20000421  
Entered Medline: 20000410

L6 ANSWER 2 OF 39 MEDLINE

TI A role for cpeYZ in cyanobacterial phycoerythrin biosynthesis.  
AB Pigment mutant strain FdR1 of the filamentous cyanobacterium *Fremyella*  
*diplosiphon* is characterized by constitutive synthesis of the  
**phycobiliprotein** phycoerythrin due to insertional inactivation of  
the *rcaC* regulatory gene by endogenous transposon Tn5469. Whereas the  
parental strain Fd33 harbors five genomic copies of Tn5469, cells of  
strain FdR1 harbor six genomic copies of the element; the sixth copy in  
FdR1 is localized to the *rcaC* gene. Electroporation of FdR1 cells yielded  
secondary pigment mutant strains FdR1E1 and FdR1E4, which identically  
exhibited the FdR1 phenotype with significantly reduced levels of  
phycoerythrin. In both FdR1E1 and FdR1E4, a seventh genomic copy of Tn5469  
was localized to the *cpeY* gene of the sequenced but phenotypically  
uncharacterized *cpeYZ* gene set. This gene set is located downstream of the  
*cpeBA* operon which encodes the alpha and beta subunits of phycoerythrin.  
Complementation experiments correlated *cpeYZ* activity to the phenotype of  
strains FdR1E1 and FdR1E4. The predicted CpeY and CpeZ proteins share  
significant sequence identity with the products of homologous *cpeY* and  
*cpeZ* genes reported for *Pseudanabaena* sp. strain PCC 7409 and  
*Synechococcus* sp. strain WH 8020, both of which synthesize phycoerythrin.  
The CpeY and CpeZ proteins belong to a family of structurally related  
cyanobacterial proteins that includes the subunits of the CpcE/CpcF  
phycocyanin alpha-subunit lyase of *Synechococcus* sp. strain PCC 7002 and  
the subunits of the PecE/PecF **phycoerythrocyanin** alpha-subunit  
lyase of *Anabaena* sp. strain PCC 7120. Phycobilisomes isolated from mutant  
strains FdR1E1 and FdR1E4 contained equal amounts of chromophorylated  
alpha and beta subunits of phycoerythrin at 46% of the levels of the  
parental strain FdR1. These results suggest that the *cpeYZ* gene products  
function in phycoerythrin synthesis, possibly as a lyase involved in the  
attachment of phycoerythrobilin to the alpha or beta subunit.

ACCESSION NUMBER: 97175521 MEDLINE  
DOCUMENT NUMBER: 97175521 PubMed ID: 9023176  
TITLE: A role for cpeYZ in cyanobacterial phycoerythrin  
biosynthesis.  
AUTHOR: Kahn K; Mazel D; Houmard J; Tandeau de Marsac N; Schaefer M  
R  
CORPORATE SOURCE: School of Biological Sciences, University of  
Missouri-Kansas City, 64110, USA.  
SOURCE: JOURNAL OF BACTERIOLOGY, (1997 Feb) 179 (4) 998-1006.  
Journal code: 2985120R. ISSN: 0021-9193.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-X04592  
ENTRY MONTH: 199703  
ENTRY DATE: Entered STN: 19970321  
Last Updated on STN: 19990129  
Entered Medline: 19970307

L6 ANSWER 3 OF 39 MEDLINE

TI Candidate genes for the **phycoerythrocyanin** alpha subunit lyase.

Biochemical analysis of pecE and pecF interposon mutants.

AB The rod substructures of the *Anabaena* sp. PCC 7120 phycobilisome contain the light harvesting proteins C-phycocyanin and **phycoerythrocyanin** (PEC). Even at low light intensities, PEC represents no more than 5% of the phycobilisome protein. The beta subunits of both proteins carry thioether-linked phycocyanobilin (PCB) at beta-Cys-82 and beta-Cys-155; however, C-phycocyanin has PCB at alpha-Cys-84 whereas PEC alpha subunit carries phycobiliviolin at this position. The *Anabaena* sp. PCC 7120 pec operon is made up of five genes. PecB and pecA encode the beta and alpha subunits of PEC, pecC encodes a linker polypeptide associated with PEC in the rod substructure, and pecE and pecF are genes of unknown function that show a high degree of homology to cpcE and cpcF, that encode a C-phycocyanin alpha subunit PCB lyase (Fairchild, C. D., Zhao, J., Zhou, J., Colson, S. E., Bryant, D. A., and Glazer, A. N. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 7017-7021). Insertional mutants in pecE and pecF, and an interposon mutant in which a portion of both pecE and pecF was deleted, were constructed. All three types of mutants grew 1.3 times slower than wild-type under limiting light conditions and showed a 20% reduction in the PCB content of whole cells relative to chlorophyll alpha. Holo-PEC was missing from the phycobilisomes of all three types of mutants and the level of the PEC linker polypeptide was reduced relative to the wild-type. However, approximately 30% of the wild-type level of the PEC beta subunit was present in all of these phycobilisomes. In contrast, the PEC alpha subunit was barely detectable in the pecE and pecF mutants, but was present in the pecEF deletion mutant as a PCB-adduct in a 1:1 ratio with the PEC beta subunit. The identity of this "unnatural" adduct was confirmed by isolation of the subunit and amino-terminal sequencing. These biochemical results support the inference that pecE and pecF encode a PEC alpha subunit phycobiliviolin lyase, and, in conjunction with earlier findings, demonstrate that **phycobiliprotein** bilin lyases show high selectivity (rather than absolute specificity) for both the bilin and the polypeptide substrate.

ACCESSION NUMBER: 95279433 MEDLINE

DOCUMENT NUMBER: 95279433 PubMed ID: 7759546

TITLE: Candidate genes for the **phycoerythrocyanin** alpha subunit lyase. Biochemical analysis of pecE and pecF interposon mutants.

AUTHOR: Jung L J; Chan C F; Glazer A N

CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, Berkeley 94720, USA.

CONTRACT NUMBER: GM28994 (NIGMS)

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 May 26) 270 (21) 12877-84.

Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199506

ENTRY DATE: Entered STN: 19950707

Last Updated on STN: 19950707

Entered Medline: 19950628

L6 ANSWER 4 OF 39 MEDLINE

TI The complete amino acid sequence of R-phycocyanin-I alpha and beta subunits from the red alga *Porphyridium cruentum*. Structural and phylogenetic relationships of the phycocyanins within the **phycobiliprotein** families.

AB We present here the complete primary structure of R-phycocyanin-I alpha and beta subunits from the red alga *Porphyridium cruentum*. The alpha chain is composed of 162 amino acid residues (18049 Da, calculated from sequence, including chromophore) and carries a phycocyanobilin pigment

covalently linked to Cys84. The beta chain contains 172 amino acids (19344Da, calculated from sequence, including chromophores) and carries a phycocyanobilin pigment covalently linked at Cys82 and a phycoerythrobilin pigment at Cys153. A gamma-N-methyl asparagine residue was also characterised at position beta 72 similar to other **phycobiliprotein** beta subunits. R-phycocyanin-I from *Porphyridium cruentum* shares high sequence identity with C-phycocyanins (69-83%), R-phycocyanins (66-70%) and in a less extent with phycoerythrocyanins (57-65%) from various sources. The presented phylogenetic trees are based on a comparison of all **phycobiliprotein** amino acid sequences known so far and confirm the clear affiliation of the R-phycocyanins in the phycocyanin family. In spite of their particular phycobilin pattern, they do not represent intermediate forms between the phycocyanin and the phycoerythrin family. **Phycoerythrocyanin**, a phycocyanin-related **phycobiliprotein** adapted to green light harvesting, is also shown to belong to the phycocyanin family. However, the phycoerythrocyanins diverge from phycocyanins in their different function and it is suggested that they should be assigned to a separate group within the phycocyanin family.

ACCESSION NUMBER: 94222105 MEDLINE  
DOCUMENT NUMBER: 94222105 PubMed ID: 8168545  
TITLE: The complete amino acid sequence of R-phycocyanin-I alpha and beta subunits from the red alga *Porphyridium cruentum*. Structural and phylogenetic relationships of the phycocyanins within the **phycobiliprotein** families.  
AUTHOR: Ducret A; Sidler W; Frank G; Zuber H  
CORPORATE SOURCE: Institute for Molecular Biology and Biophysics, Federal Institute of Technology, Zurich, Switzerland.  
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1994 Apr 1) 221 (1) 563-80.  
Journal code: 0107600. ISSN: 0014-2956.  
PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199406  
ENTRY DATE: Entered STN: 19940613  
Last Updated on STN: 19940613  
Entered Medline: 19940602

L6 ANSWER 5 OF 39 MEDLINE

TI Structure of the genes encoding the rod-core linker polypeptides of *Mastigocladus laminosus* phycobilisomes and functional aspects of the **phycobiliprotein**/linker-polypeptide interactions.

AB The 3' portion of the *cpc* operon in *Mastigocladus laminosus* encloses the genes 5'-*cpcF*-*cpcG1*-*cpcG2*-*cpcG3* 3'. The three *cpcG* genes encode different phycocyanin-associated rod-core linker polypeptides of the phycobilisomes with predicted 279, 247 and 254 amino acids in length. The gene products *CpcG* show a high similarity at their N-terminal domains (190 amino acids) and an overall identity of 47-53% to one another. Each of the three *CpcG* polypeptides is highly related to one of the four *CpcG* gene products of *Anabaena* sp. PCC 7120 (66-81% identity). It is suggested that these pairs of rod-core linker polypeptides mediate the same specific type of phycocyanin----allophycocyanin interaction in the similar phycobilisomes of *M. laminosus* and *Anabaena* sp. PCC 7120. The similarity of the *CpcG1*, *CpcG2* and *CpcG3* polypeptides to the single *CpcG* rod-core linker polypeptide of *Synechococcus* sp. PCC 7002 (36-41% identity) is lower. The rod-core linker polypeptides are more distantly related to the rod linker polypeptides associated with phycocyanin or phycoerythrin. However, six conserved domains were identified within the N-terminal 190 amino acids of these linker proteins, which bear similar amino acid sequences, including highly conserved basic amino acids. A similar amino acid sequence but with conserved acidic amino acids can be found in the beta subunits of

phycocyanin, phycoerythrin and **phycoerythrocyanin**, which is protruding into the central cavity of the **phycobiliprotein** hexamers. It is suggested that these domains are sites of **phycobiliprotein**-hexamer/rod and rod-core linker interactions.

ACCESSION NUMBER: 92249337 MEDLINE  
DOCUMENT NUMBER: 92249337 PubMed ID: 1577010  
TITLE: Structure of the genes encoding the rod-core linker polypeptides of *Mastigocladus laminosus* phycobilisomes and functional aspects of the **phycobiliprotein** /linker-polypeptide interactions.  
AUTHOR: Glauser M; Stirewalt V L; Bryant D A; Sidler W; Zuber H  
CORPORATE SOURCE: Institut fur Molekularbiologie und Biophysik, Eidgenossische Technische Hochschule, Zurich, Switzerland.  
CONTRACT NUMBER: GM-31625 (NIGMS)  
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1992 May 1) 205 (3) 927-37.  
Journal code: 0107600. ISSN: 0014-2956.  
PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-S87180; GENBANK-S87218; GENBANK-S87221; GENBANK-S87223; GENBANK-X59763; GENBANK-X64458; GENBANK-X65178; GENBANK-X65179; GENBANK-X65180; GENBANK-X65181  
ENTRY MONTH: 199206  
ENTRY DATE: Entered STN: 19920619  
Last Updated on STN: 19920619  
Entered Medline: 19920609

L6 ANSWER 6 OF 39 MEDLINE

TI Refined three-dimensional structure of **phycoerythrocyanin** from the cyanobacterium *Mastigocladus laminosus* at 2.7 A.

AB The structure of the **phycobiliprotein phycoerythrocyanin** from the thermophilic cyanobacterium *Mastigocladus laminosus* has been determined at 2.7 A resolution by X-ray diffraction methods on the basis of the molecular model of C-phycocyanin from the same organism. Hexagonal **phycoerythrocyanin** crystals of space group P6(3) with cell constants  $a = b = 156.86$  A,  $c = 40.39$  A,  $\alpha = \beta = 90$  degrees,  $\gamma = 120$  degrees are almost isomorphous to C-phycocyanin crystals. The crystal structure has been refined by energy-restrained crystallographic refinement and model building. The conventional crystallographic R-factor of the final model was 19.2% with data to 2.7 A resolution. In **phycoerythrocyanin**, the three ( $\alpha$   $\beta$ )-subunits are arranged around a 3-fold symmetry axis, as in C-phycocyanin. The two structures are very similar. After superposition, the 162 C  $\alpha$  atoms of the  $\alpha$ -subunit have a mean difference of 0.71 A and the 171 C  $\alpha$  atoms of the  $\beta$ -subunit differ by 0.51 A. The stereochemistry of the chiral atoms in the phycobiliviolin chromophore A84 is C(31)-R, C(4)-S. The configuration of the chromophore is C(10)-Z, C(15)-Z and the conformation C(5)-anti, C(9)-syn and C(14)-anti like the phycocyanobilin chromophores in **phycoerythrocyanin** and C-phycocyanin.

ACCESSION NUMBER: 90172426 MEDLINE  
DOCUMENT NUMBER: 90172426 PubMed ID: 2106585  
TITLE: Refined three-dimensional structure of **phycoerythrocyanin** from the cyanobacterium *Mastigocladus laminosus* at 2.7 A.  
AUTHOR: Duerring M; Huber R; Bode W; Ruembeli R; Zuber H  
CORPORATE SOURCE: Max-Planck Institut fur Biochemie, Martinsried, Germany.  
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1990 Feb 5) 211 (3) 633-44.  
Journal code: 2985088R. ISSN: 0022-2836.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English

FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199004  
ENTRY DATE: Entered STN: 19900601  
Last Updated on STN: 19900601  
Entered Medline: 19900409

L6 ANSWER 7 OF 39 MEDLINE

TI Crosslinking of phycobiliproteins from the cyanobacterium *Mastigocladus laminosus* with bis-imidates: localization of an intrasubunit and an intersubunit crosslink in C-phycocyanin.

AB The light-harvesting pigment-protein complexes allophycocyanin (AP), C-phycocyanin (PC) and **phycoerythrocyanin** (PEC) of the cyanobacterium *Mastigocladus laminosus* consist of alpha- and beta-subunits containing about 170 amino-acid residues each. These two subunits form an alpha,beta-monomer, three of which build up a disc-shaped trimer. In this study these phycobiliproteins were crosslinked with bis-imidates. Various spacer lengths of the reagent and various aggregation states of the **phycobiliprotein** were tested. An intersubunit crosslink could be verified in all three phycobiliproteins. PC-trimers were crosslinked with the homobifunctional reagent dimethyl pimelimidate having a maximal crosslinking distance of 10 A. Two crosslinks could be identified: an intramonomer intersubunit crosslink with a yield of 48% and an intrasubunit crosslink within alpha PC (57%). These products were chemically and enzymatically fragmented and the small crosslinked peptides were isolated and then identified by amino-acid analysis. The following amino acids were crosslinked: alpha-Val 1 with beta-Ala 1 and alpha-Lys 62 with alpha-Lys 134. Both crosslinks could be localized within the known three-dimensional structure of PC.

ACCESSION NUMBER: 88050102 MEDLINE

DOCUMENT NUMBER: 88050102 PubMed ID: 3118901

TITLE: Crosslinking of phycobiliproteins from the cyanobacterium *Mastigocladus laminosus* with bis-imidates: localization of an intrasubunit and an intersubunit crosslink in C-phycocyanin.

AUTHOR: Rumbeli R; Wirth M; Zuber H

CORPORATE SOURCE: Institut fur Mokekularbiologie und Biophysik, Eidgenossische Technische Hochschule, Zurich.

SOURCE: BIOLOGICAL CHEMISTRY HOPPE-SEYLER, (1987 Sep) 368 (9) 1179-91.

Journal code: 8503054. ISSN: 0177-3593.

PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 198801

ENTRY DATE: Entered STN: 19900305

Last Updated on STN: 19900305

Entered Medline: 19880115

L6 ANSWER 8 OF 39 MEDLINE

TI Linker polypeptides of the phycobilisome from the cyanobacterium *Mastigocladus laminosus*. II. Amino-acid sequences and functions.

AB The complete primary structure of an 80-residue linker polypeptide, LR(C)8.9, from the phycobilisome of the cyanobacterium *Mastigocladus laminosus* was determined as well as the 44 N-terminal residues of the two linker polypeptides LR34.5,PEC and LR34.5,PC and the 114 C-terminal residues of LR34.5,PEC. A brief description of the structure determination and an extensive discussion of the relationships of these polypeptides have been published recently (Fuglistaller, P., Suter, F. & Zuber, H. (1985) Biol. Chem. Hoppe-Seyler 366, 993-1001). In this paper we report in detail about the elucidation of the primary structures. Limited digestion of the hexameric **phycobiliprotein**-linker polypeptide complex (alpha PEC beta PEC)6LR34.5,PEC with various proteases resulted in a linker polypeptide diminished by a 1-5 kDa segment, while the

phycobiliproteins remained intact. By N-terminal sequence analysis of the residual part of the linker polypeptide in the complex, LR34.5,PEC, it was concluded that the C-terminus of the polypeptide had been attacked by the proteases. This C-terminal part of the protein influences the hexamer formation of **phycoerythrocyanin** (PEC) and is responsible for the linkage between two **phycobiliprotein** hexamers. From the function of the C-terminal segment of LR34.5,PEC and its homology to the LR(C)8.9 polypeptide, it was concluded that LR(C)8.9 is located at the end of the peripheral phycobilisomal rods distal to the allophycocyanin core.

ACCESSION NUMBER: 87000168 MEDLINE  
 DOCUMENT NUMBER: 87000168 PubMed ID: 3092841  
 TITLE: Linker polypeptides of the phycobilisome from the cyanobacterium *Mastigocladus laminosus*. II. Amino-acid sequences and functions.  
 AUTHOR: Fuglistaller P; Suter F; Zuber H  
 SOURCE: BIOLOGICAL CHEMISTRY HOPPE-SEYLER, (1986 Jul) 367 (7) 615-26.  
 Journal code: 8503054. ISSN: 0177-3593.  
 PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 198611  
 ENTRY DATE: Entered STN: 19900302  
 Last Updated on STN: 19900302  
 Entered Medline: 19861114

L6 ANSWER 9 OF 39 MEDLINE

TI Linker polypeptides of the phycobilisome from the cyanobacterium *Mastigocladus laminosus*. I. Isolation and characterization of **phycobiliprotein**-linker-polypeptide complexes.

AB Phycobilisomes from the cyanobacterium *Mastigocladus laminosus* cultured in white and red light were isolated and compared with respect to the **phycoerythrocyanin** (PEC) and linker polypeptide contents. It was verified that the production of PEC is induced by low light intensities. A PEC complex, (alpha PEC beta PEC)6LR34.5,PEC, and a phycocyanin (PC) complex, (alpha PC beta PC)6LR34.5,PC, were isolated from phycobilisomes by Cellex-D anion exchange chromatography and sucrose density gradient centrifugation. The absorption and fluorescence emission maxima of the PEC complex are at 575 and 620 nm and those of the PC complex are at 631 and 647 nm, respectively. The extinction coefficients of the two complexes were determined. From different experiments it was concluded that PEC is present as a hexameric complex, (alpha PEC beta PEC)6LR34.5,PEC, in the phycobilisome. The two linker polypeptides LR34.5,PEC and LR34.5,PC were isolated from their **phycobiliprotein** complexes by gel filtration on Bio-Gel P-100 in 50% formic acid. A 5-kDa terminal segment of both linker polypeptides was found to influence the hexamer formation of the phycobiliproteins. The same segments have been described to be responsible for the hexamer-hexamer linkage (Yu, M.-H. & Glazer, A.N. (1982) J. Biol. Chem. 257, 3429-3433). A 8.9-kDa linker polypeptide, LR(C)8.9, was isolated from a PEC fraction of the Cellex-D column by Bio-Gel P-100 gel filtration in 50% formic acid. Localisation of this protein within the phycobilisome was attempted. Its most probable function is to terminate the phycobilisomal rods at the end distal to the allophycocyanin core.

ACCESSION NUMBER: 87000167 MEDLINE  
 DOCUMENT NUMBER: 87000167 PubMed ID: 3092840  
 TITLE: Linker polypeptides of the phycobilisome from the cyanobacterium *Mastigocladus laminosus*. I. Isolation and characterization of **phycobiliprotein**-linker-polypeptide complexes.  
 AUTHOR: Fuglistaller P; Suter F; Zuber H  
 SOURCE: BIOLOGICAL CHEMISTRY HOPPE-SEYLER, (1986 Jul) 367 (7) 601-14.  
 Journal code: 8503054. ISSN: 0177-3593.

PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198611  
ENTRY DATE: Entered STN: 19900302  
Last Updated on STN: 19900302  
Entered Medline: 19861114

L6 ANSWER 10 OF 39 MEDLINE

TI Linker polypeptides of the phycobilisome from the cyanobacterium  
Mastigocladus laminosus: amino-acid sequences and relationships.  
AB Three linker polypeptides of the phycobilisome from the cyanobacterium  
Mastigocladus laminosus were isolated: A 8.9-kDa polypeptide, L8.9R(C),  
which is probably associated with C-phycocyanin, a 34.5-kDa polypeptide,  
L34.5,PCR, which forms a complex with C-phycocyanin, and a 34.5-kDa  
polypeptide, L34.5,PECR, which is linked to **phycoerythrocyanin**.  
The complete amino-acid sequence (80 residues) of the L8.9R(C) polypeptide  
was determined as well as the N-terminal 44 residues of both L34.5R  
polypeptides and the 114 C-terminal residues of L34.5,PECR. L8.9R(C) is  
homologous to L8.9C (Fuglistaller et al. (1984) Hoppe-Seyler's Z. Physiol.  
Chem. 365, 1085-1096) and to the C-terminal sequence of L34.5,PECR. The  
N-terminal sequences of L34.5,PECR and L34.5,PCR exhibit 34% homology. The  
44 N-terminal residues of L34.5,PECR are related to the beta-subunit of  
**phycoerythrocyanin** (23% homology), while the C-terminal sequence  
of L34.5,PECR is more related to alpha PEC (21% homology within 60  
residues). This suggests that the 30-kDa-linker polypeptide family  
originates from a fusion of the alpha- and beta-subunit genes and the  
corresponding intercistronic DNA sequence, as might have arisen through  
mutation in the stop-codon of the beta-subunit gene. Hence, all  
polypeptides of the phycobilisome (including perhaps the anchor  
polypeptide) may be derived from an early ancestor  
**phycobiliprotein** subunit, which itself is also related to  
myoglobin (Schirmer et al. (1985) J. Mol. Biol. 184, 251-277).

ACCESSION NUMBER: 86050914 MEDLINE

DOCUMENT NUMBER: 86050914 PubMed ID: 3933528

TITLE: Linker polypeptides of the phycobilisome from the  
cyanobacterium Mastigocladus laminosus: amino-acid  
sequences and relationships.

AUTHOR: Fuglistaller P; Suter F; Zuber H

SOURCE: BIOLOGICAL CHEMISTRY HOPPE-SEYLER, (1985 Oct) 366 (10)  
993-1001.

Journal code: 8503054. ISSN: 0177-3593.

PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 198601

ENTRY DATE: Entered STN: 19900321

Last Updated on STN: 19900321

Entered Medline: 19860117

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L6 ANSWER 11 OF 39 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

TI Characterization of phycoviolobilin **phycoerythrocyanin**  
-alpha84-cystein-lyase-(isomerizing) from Mastigocladus laminosus.

AB Cofactor requirements and enzyme kinetics have been studied of the novel,  
dual-action enzyme, the isomerizing phycoviolobilin  
**phycoerythrocyanin**-alpha84-cystein-lyase (PVB-PEC-lyase) from  
Mastigocladus laminosus, which catalyses both the covalent attachment of  
phycocyanobilin to PecA, the apo-alpha-subunit of  
**phycoerythrocyanin**, and its isomerization to phycoviolobilin.  
Thiols and the divalent metals, Mg<sup>2+</sup> or Mn<sup>2+</sup>, were required, and the  
reaction was aided by the detergent, Triton X-100. Phosphate buffer  
inhibits precipitation of the proteins present in the reconstitution

mixture, but at the same time binds the required metal. Kinetic constants were obtained for both substrates, the chromophore ( $K_m=12-16 \mu M$ , depending on (PecA),  $k_{cat} \approx 1.2 \times 10^{-4} \text{ s}^{-1}$ ) and the apoprotein ( $K_m=2.4 \mu M$  at  $14 \mu M$  PCB,  $k_{cat}=0.8 \times 10^{-4} \text{ s}^{-1}$ ). The kinetic analysis indicated that the reconstitution reaction proceeds by a sequential mechanism. By a combination of untagged and His-tagged subunits, evidence was obtained for a complex formation between PecE and PecF (subunits of PVB-PEC-lyase), and by experiments with single subunits for the prevalent function of PecE in binding and PecF in isomerizing the chromophore.

ACCESSION NUMBER: 2002:561644 BIOSIS  
DOCUMENT NUMBER: PREV200200561644  
TITLE: Characterization of phycoviolobilin  
**phycocerythrocyanin**-alpha84-cystein-lyase-  
(isomerizing) from *Mastigocladus laminosus*.  
AUTHOR(S): Zhao, Kai-Hong (1); Wu, Dong; Wang, Lu; Zhou, Ming; Storf, Max; Bubenzer, Claudia; Strohmann, Brigitte; Scheer, Hugo  
CORPORATE SOURCE: (1) College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hubei, 430074: khzhao@163.com, scheer-h@botanik.biologie.uni-muenchen.de China  
SOURCE: European Journal of Biochemistry, (September, 2002) Vol. 269, No. 18, pp. 4542-4550. <http://www.blackwell-science.com/cgiilib/jnlpage.asp?journal=ejb&file=ejb&page=aims.print>.  
ISSN: 0014-2956.  
DOCUMENT TYPE: Article  
LANGUAGE: English

L6 ANSWER 12 OF 39 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
TI One century of protein crystallography: The phycobiliproteins.  
AB The physical principles that underlay the rapid and efficient energy transfer from the light absorbing phycobilisomes to the reaction centre are conceivable from the knowledge of the exact three-dimensional structure of the phycobiliproteins and chromophores that are involved. The structure of the components and their assembly in the phycobilisomes could be determined by the structure analysis of X-ray data derived from **phycobiliprotein** crystals. Reports about these very aesthetic and brilliantly colored crystals have been published for more than a hundred years but it was only in the last decade that the structures of the different members of the **phycobiliprotein** family were solved for the first time at atomic resolution - all of them in Martinsried at the Max-Planck-Institut fur Biochemie. Despite the appearance of common structural principles the most important finding was that very subtle modifications in the structure and environment of the chromophores are sufficient to establish a highly specific light harvesting system in which the phycobiliproteins function with great cooperativity and efficiency.

ACCESSION NUMBER: 1997:290832 BIOSIS  
DOCUMENT NUMBER: PREV199799590035  
TITLE: One century of protein crystallography: The phycobiliproteins.  
AUTHOR(S): Betz, Michael  
CORPORATE SOURCE: Max-Planck-Institut fuer Biochemie, Abteilung Strukturforschung, Am Klopferspitz 18a, D-82152 Martinsried Germany  
SOURCE: Biological Chemistry, (1997) Vol. 378, No. 3-4, pp. 167-176.  
ISSN: 1431-6730.  
DOCUMENT TYPE: General Review  
LANGUAGE: English

L6 ANSWER 13 OF 39 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
TI A role for cpeYZ in cyanobacterial phycocerythrin biosynthesis.  
AB Pigment mutant strain FdR1 of the filamentous cyanobacterium *Fremyella diplosiphon* is characterized by constitutive synthesis of the

**phycobiliprotein** phycoerythrin due to insertional inactivation of the *rcaC* regulatory gene by endogenous transposon Tn5469. Whereas the parental strain Fd33 harbors five genomic copies of Tn5469, cells of strain FdR1 harbor six genomic copies of the element; the sixth copy in FdR1 is localized to the *rcaC* gene. Electroporation of FdR1 cells yielded secondary pigment mutant strains FdR1E1 and FdR1E4, which identically exhibited the FdR1 phenotype with significantly reduced levels of phycoerythrin. In both FdR1E1 and FdR1E4, a seventh genomic copy of Tn5469 was localized to the *cpeY* gene of the sequenced but phenotypically uncharacterized *cpeYZ* gene set. This gene set is located downstream of the *cpeBA* operon which encodes the alpha and beta subunits of phycoerythrin. Complementation experiments correlated *cpeYZ* activity to the phenotype of strains FdR1E1 and FdR1E4. The predicted CpeY and CpeZ proteins share significant sequence identity with the products of homologous *cpeY* and *cpeZ* genes reported for *Pseudanabaena* sp. strain PCC 7409 and *Synechococcus* sp. strain WH 8020, both of which synthesize phycoerythrin. The CpeY and CpeZ proteins belong to a family of structurally related cyanobacterial proteins that includes the subunits of the CpcE/CpcF phycocyanin alpha-subunit lyase of *Synechococcus* sp. strain PCC 7002 and the subunits of the PecE/PecF **phycoerythrocyanin** alpha-subunit lyase of *Anabaena* sp. strain PCC 7120. Phycobilisomes isolated from mutant strains FdR1E1 and FdR1E4 contained equal amounts of chromophorylated alpha and beta subunits of phycoerythrin at 46% of the levels of the parental strain FdR1. These results suggest that the *cpeYZ* gene products function in phycoerythrin synthesis, possibly as a lyase involved in the attachment of phycoerythrobilin to the alpha or beta subunit.

ACCESSION NUMBER: 1997:155769 BIOSIS  
DOCUMENT NUMBER: PREV199799454972  
TITLE: A role for *cpeYZ* in cyanobacterial phycoerythrin biosynthesis.  
AUTHOR(S): Kahn, Katherine; Mazel, Didier; Houmard, Jean; De Marsac, Nicole Tandeau; Schaefer, Michael R. (1)  
CORPORATE SOURCE: (1) Univ. Missouri-Kansas City, Sch. Biol. Sci., 5100 Rockhill Rd., Kansas City, MO 64110 USA  
SOURCE: Journal of Bacteriology, (1997) Vol. 179, No. 4, pp. 998-1006.  
ISSN: 0021-9193.  
DOCUMENT TYPE: Article  
LANGUAGE: English

L6 ANSWER 14 OF 39 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

TI Candidate genes for the **phycoerythrocyanin** alpha subunit lyase: Biochemical analysis of *pecE* and *pecF* interposon mutants.

AB The rod substructures of the *Anabaena* sp. PCC 7120 phycobilisome contain the light harvesting proteins C-phycocyanin and **phycoerythrocyanin** (PEC). Even at low light intensities, PEC represents no more than 5% of the phycobilisome protein. The beta subunits of both proteins carry thioether-linked phycocyanobilin (PCB) at beta-Cys-82 and beta-Cys-155; however, C-phycocyanin has PCB at alpha-Cys-84 whereas PEC a subunit carries phycobiliviolin at this position. The *Anabaena* sp. PCC 7120 *pec* operon is made up of five genes. *PecB* and *pecA* encode the beta and alpha subunits of PEC, *pecC* encodes a linker polypeptide associated with PEC in the rod substructure, and *pecE* and *pecF* are genes of unknown function that show a high degree of homology to *cpcE* and *cpcF*, that encode a C-phycocyanin a subunit PCB lyase (Fairchild, C. D., Zhao, J., Zhou, J., Colson, S. E., Bryant, D. A., and Glazer, A. N. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7017-7021). Insertional mutants in *pecE* and *pecF*, and an interposon mutant in which a portion of both *pecE* and *pecF* was deleted, were constructed. All three types of mutants grew 1.3 times slower than wild-type under limiting light conditions and showed a 20% reduction in the PCB content of whole cells relative to chlorophyll alpha. Holo-PEC was missing from the phycobilisomes of all three types of mutants and the level of the PEC linker polypeptide was reduced relative to the wild-type. However, approx 30% of the wild-type level of the PEC beta subunit was

present in all of these phycobilisomes. In contrast, the PEC  $\alpha$  subunit was barely detectable in the *pecE* and *pecF* mutants, but was present in the *pecEF* deletion mutant as a PCB-adduct in a 1:1 ratio with the PEC  $\beta$  subunit. The identity of this "unnatural" adduct was confirmed by isolation of the subunit and amino-terminal sequencing. These biochemical results support the inference that *pecE* and *pecF* encode a PEC  $\alpha$  subunit phycobiliviolin lyase, and, in conjunction with earlier findings, demonstrate that **phycobiliprotein** bilin lyases show high selectivity (rather than absolute specificity) for both the bilin and the polypeptide substrate.

ACCESSION NUMBER: 1995:320261 BIOSIS  
DOCUMENT NUMBER: PREV199598334561  
TITLE: Candidate genes for the **phycoerythrocyanin**  $\alpha$  subunit lyase: Biochemical analysis of *pecE* and *pecF* interposon mutants.  
AUTHOR(S): Jung, Linda J.; Chan, Crystal F.; Glazer, Alexander N. (1)  
CORPORATE SOURCE: (1) MCB: Stanley/Donner ASU, 229 Stanley Hall 3206, Univ. Calif., Berkeley, CA 94720-3206 USA  
SOURCE: Journal of Biological Chemistry, (1995) Vol. 270, No. 21, pp. 12877-12884.  
ISSN: 0021-9258.  
DOCUMENT TYPE: Article  
LANGUAGE: English

L6 ANSWER 15 OF 39 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
TI Type I and type II reversible photochemistry of **phycoerythrocyanin**  $\alpha$ -subunit from *Mastigocladus laminosus* both involve Z, E isomerization of phycoviolobilin chromophore and are controlled by sulfhydryls in apoprotein.  
AB The  $\alpha$ -subunit of **phycoerythrocyanin** ( $\alpha$ -PEC) can exist in four states (Z- $\alpha$ -I, Z- $\alpha$ -II, E- $\alpha$ -I, E- $\alpha$ -II). They are connected pairwise by photoreversible photochromism. The type I photochemistry connecting Z- $\alpha$ -I and E- $\alpha$ -I, involves a 15Z/E phototransformation.  $\alpha$ -PEC showing this type of photochemistry is obtained when the subunits of PEC are separated by gel permeation chromatography in the presence of 63 mM formic acid, or by reduction of the  $\alpha$ -subunit of **phycoerythrocyanin** of type II reversible photochemistry with mercaptoethanol.  $\alpha$ -PEC showing the recently characterized (Hong et al. (1993) Photochem. Photobiol. 58, 745-747) type II photochemistry connecting Z- $\alpha$ -II and E- $\alpha$ -II can be obtained when the  $\alpha$ -subunit of **phycoerythrocyanin** of type I photochemistry is allowed to oxidize, or when it is treated with p-chloromercuribenzenesulfonate. The two types of reversible photochemistry of  $\alpha$ -subunit of **phycoerythrocyanin** are therefore controlled by the state of the two sulfhydryl group(s), viz. Cys-98,99 of the apoprotein. A quantitative analysis of the PCMS titration showed that modification of either one of these two cysteine residues is sufficient to inhibit type I photochemistry and induces type II. By treatment with mercaptoethanol or PCMS, the end products of type I and type II photochemistry, respectively, could be pairwise transformed into each other, showing that type II also involves 15Z/E isomerization. The difference between them must be due to different interactions between phycoviolobilin and apoprotein, which can be modulated by the two sulfhydryls.

ACCESSION NUMBER: 1995:226654 BIOSIS  
DOCUMENT NUMBER: PREV199598240954  
TITLE: Type I and type II reversible photochemistry of **phycoerythrocyanin**  $\alpha$ -subunit from *Mastigocladus laminosus* both involve Z, E isomerization of phycoviolobilin chromophore and are controlled by sulfhydryls in apoprotein.  
AUTHOR(S): Zhao, Kai-Hong; Scheer, Hugo (1)  
CORPORATE SOURCE: (1) Botanisches Inst. Univ., Menzinger Str. 67, D-80623, Muenchen Germany

SOURCE: Biochimica et Biophysica Acta, (1995) Vol. 1228, No. 2-3,  
pp. 244-253.  
ISSN: 0006-3002.  
DOCUMENT TYPE: Article  
LANGUAGE: English

=> d his

(FILE 'HOME' ENTERED AT 15:16:17 ON 28 MAR 2003)

FILE 'MEDLINE, BIOSIS, EMBASE, DGENE, WPIDS, FSTA, JICST-EPLUS, JAPIO'  
ENTERED AT 15:17:25 ON 28 MAR 2003

L1 143 S PHYCOERYTHROCYANIN  
L2 9 S HOLO ALPHA SUBUNIT  
L3 3 S L2 AND L1  
L4 21 S PHYCOBILIVIOLIN  
L5 724 S PHYCOBILIPROTEIN  
L6 39 S L1 AND L5  
L7 1 S L6 AND L2

=> d l6 ti abs ibib 30-39

L6 ANSWER 30 OF 39 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
TI Biosynthesis of the cyanobacterial light-harvesting polypeptide  
**phycoerythrocyanin** holo-.alpha. subunit in a heterologous host.  
AB The entire pathway for the biosynthesis of the phycobiliviolin-bearing  
His-tagged holo-.alpha. subunit of the cyanobacterial photosynthetic  
accessory protein **phycoerythrocyanin** was reconstituted in  
Escherichia coli. Cyanobacterial genes encoding enzymes required for the  
conversion of heme to 3Z-phycocyanobilin, a precursor of phycobiliviolin  
(namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin  
oxidoreductase), were expressed from a plasmid under the control of the  
hybrid trp-lac (trc) promoter. Genes for the apo-  
**phycoerythrocyanin** .alpha. subunit (pecA) and the heterodimeric  
lyase/isomerase (pecE and pecF), which catalyzes both the covalent  
attachment of phycocyanobilin and its concurrent isomerization to  
phycobiliviolin, were expressed from the trc promoter on a second plasmid.  
Upon induction, recombinant E. coli used endogenous heme to produce  
holo-PecA with absorbance and fluorescence properties similar to those of  
the same protein produced in cyanobacteria. About two-thirds of the  
apo-PecA was converted to holo-PecA. No significant bilin addition took  
place in a similarly engineered E. coli strain that lacks pecE and pecF.  
By using immobilized metal affinity chromatography, both apo-PecA and  
holo-PecA were isolated as ternary complexes with PecE and PecF. The  
identities of all three components in the ternary complexes were  
established unambiguously by protein and tryptic peptide analyses  
performed by matrix-assisted laser desorption ionization-time of flight  
mass spectrometry.

ACCESSION NUMBER: 2002295599 EMBASE  
TITLE: Biosynthesis of the cyanobacterial light-harvesting  
polypeptide **phycoerythrocyanin** holo-.alpha.  
subunit in a heterologous host.  
AUTHOR: Tooley A.J.; Glazer A.N.  
CORPORATE SOURCE: A.N. Glazer, Natural Reserve System, University of  
California, 1111 Franklin Street, Oakland, CA 94607-5200,  
United States. glazer@uclink4.berkeley.edu  
SOURCE: Journal of Bacteriology, (2002) 184/17 (4666-4671).  
Refs: 22  
ISSN: 0021-9193 CODEN: JOBAAY  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English

SUMMARY LANGUAGE: English

L6 ANSWER 31 OF 39 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
TI Chromophore attachment to biliproteins: Specificity of PecE/PecF, a lyase-isomerase for the photoactive 3(1)-Cys-.alpha.84-phycoviolobilin chromophore of **phycoerythrocyanin**.  
AB PecE and PecF, the products of two **phycoerythrocyanin** lyase genes (pecE and pecF) of *Mastigocladus laminosus* (Fischerella), catalyze two reactions: (1) the regiospecific addition of phycocyanobilin (PCB) to Cys-.alpha.84 of the **phycoerythrocyanin** .alpha.-subunit (PecA), and (2) the .DELTA.4.fwdarw..DELTA.2 isomerization of the PCB to the phycoviolobilin (PVB)-chromophore [Zhao et al. (2000) FEBS Lett. 469, 9-13]. The .alpha.-apoprotein (PecA) as well PecE and PecF were overexpressed from two strains of *M. laminosus*, with and without His-tags. The products of the spontaneous addition of PCB to PecA, and that of the reaction catalyzed by PecE/F, were characterized by their photochemistry and by absorption, fluorescence, circular dichroism of the four states obtained by irradiation with light (15-Z/E isomers of the chromophore) and/or modification of Cys-.alpha.98/99 with thiol-directed reagents. The spontaneous addition leads to a 3(1)-Cys-PCB adduct, which is characteristic of allophycocyanins and phycocyanins, while the addition catalyzed by PecE and PecF leads to a 3(1)-Cys-PVB adduct which after purification was identical to .alpha.-PEC. The specificity and kinetics of the chromophore additions were investigated with respect to the structure of the bilin substrate: The 3-ethylidene-bilins, viz., PCB, its 18-vinyl analogue phytochromobilin, phycoerythrobilin and its dimethylester, react spontaneously to yield the conventional addition products (3-H, 3(1)-Cys), while the 3-vinyl-substituted bilins, viz., bilirubin and biliverdin, were inactive. Only phycocyanobilin and phytochromobilin are substrates to the addition-isomerization reaction catalyzed by PecE/F. The slow spontaneous addition of phycoerythrobilin is not influenced, and there is in particular no catalyzed isomerization to urobilin.

ACCESSION NUMBER: 2001363892 EMBASE  
TITLE: Chromophore attachment to biliproteins: Specificity of PecE/PecF, a lyase-isomerase for the photoactive 3(1)-Cys-.alpha.84-phycoviolobilin chromophore of **phycoerythrocyanin**.  
AUTHOR: Storf M.; Parbel A.; Meyer M.; Strohmamm B.; Scheer H.; Deng M.-G.; Zheng M.; Zhou M.; Zhao K.-H.  
CORPORATE SOURCE: H. Scheer, Botanisches Institut, Universitat Munchen, Menzinger Strasse 67, D-80638 Munchen, Germany. scheer-h@botanik.biologie.uni-muenchen.de  
SOURCE: Biochemistry, (16 Oct 2001) 40/41 (12444-12456). Refs: 88  
ISSN: 0006-2960 CODEN: BICHAW  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 029 Clinical Biochemistry  
LANGUAGE: English  
SUMMARY LANGUAGE: English

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L6 ANSWER 32 OF 39 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
TI Novel activity of a **phycobiliprotein** lyase: Both the attachment of phycocyanobilin and the isomerization to phycoviolobilin are catalyzed by the proteins PecE and PecF encoded by the **phycoerythrocyanin** operon.  
AB The structure of phycoviolobilin, the photoactive chromophore of .alpha.-**phycoerythrocyanin**, is incompatible with a chromophore ligation to the apoprotein via SH-addition (cysteine) to a .DELTA.3,31-double bond of the phycobilin. The two putative **phycoerythrocyanin** lyase genes of *Mastigocladus laminosus*, pecE and pecF, were overexpressed in *Escherichia coli*. Their action has been studied on the addition reaction of phycocyanobilin to apo-.alpha.-**phycoerythrocyanin** (PecA). In the absence of the components of .alpha.-PEC-phycoviolobilin lyase PecE

and PecF, or in the presence of only one of them, phycocyanobilin binds covalently to PecA forming a fluorescent chromoprotein with a red-shifted absorption ( $\lambda_{\text{max}}$ =641 nm) and low photoactivity (<10%). In the presence of both PecE and PecF, a chromoprotein forms which by its absorption ( $\lambda_{\text{max}}$ =565 nm) and high photoreversible photochromism (100% type I) has been identified as integral  $\alpha$ -

**phycoerythrocyanin**. We conclude that PecE and PecF jointly catalyze not only the addition of phycocyanobilin to PecA, but also its isomerization to the native phycoviolobilin chromophore. Copyright (C) 2000 Federation of European Biochemical Societies.

ACCESSION NUMBER: 2000081098 EMBASE  
 TITLE: Novel activity of a **phycobiliprotein** lyase: Both the attachment of phycocyanobilin and the isomerization to phycoviolobilin are catalyzed by the proteins PecE and PecF encoded by the **phycoerythrocyanin** operon.  
 AUTHOR: Zhao K.-H.; Deng M.-G.; Zheng M.; Zhou M.; Parbel A.; Storf M.; Meyer M.; Strohmam B.; Scheer H.  
 CORPORATE SOURCE: K.-H. Zhao, College of Life Sciences, Wuhan University, Wuhan 430072, China. scheer-h@botanik.biologie.uni-muenchen.de  
 SOURCE: FEBS Letters, (3 Mar 2000) 469/1 (9-13).  
 Refs: 41  
 ISSN: 0014-5793 CODEN: FEBLAL  
 PUBLISHER IDENT.: S 0014-5793(00)01245-X  
 COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article  
 FILE SEGMENT: 004 Microbiology  
 LANGUAGE: English  
 SUMMARY LANGUAGE: English

L6 ANSWER 33 OF 39 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
 TI One century of protein crystallography: The phycobiliproteins.  
 AB The physical principles that underlay the rapid and efficient energy transfer from the light absorbing phycobilisomes to the reaction centre are conceivable from the knowledge of the exact three-dimensional structure of the phycobiliproteins and chromophores that are involved. The structure of the components and their assembly in the phycobilisomes could be determined by the structure analysis of X-ray data derived from **phycobiliprotein** crystals. Reports about these very aesthetic and brilliantly colored crystals have been published for more than a hundred years but it was only in the last decade that the structures of the different members of the **phycobiliprotein** family were solved for the first time at atomic resolution - all of them in Martinsried at the Max-Planck-Institut fur Biochemie. Despite the appearance of common structural principles the most important finding was that very subtle modifications in the structure and environment of the chromophores are sufficient to establish a highly specific light harvesting system in which the phycobiliproteins function with great cooperativity and efficiency.

ACCESSION NUMBER: 97164179 EMBASE  
 DOCUMENT NUMBER: 1997164179  
 TITLE: One century of protein crystallography: The phycobiliproteins.  
 AUTHOR: Betz M.  
 CORPORATE SOURCE: M. Betz, Max-Planck-Institut fur Biochemie, Abteilung Strukturforschung, Am Klopferspitz 18a, D-82152 Martinsried, Germany  
 SOURCE: Biological Chemistry, (1997) 378/3-4 (167-176).  
 Refs: 65  
 ISSN: 1431-6730 CODEN: BICHF3  
 COUNTRY: Germany  
 DOCUMENT TYPE: Journal; General Review  
 FILE SEGMENT: 004 Microbiology  
 029 Clinical Biochemistry  
 LANGUAGE: English

SUMMARY LANGUAGE: English

L6 ANSWER 34 OF 39 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI A role for cpeYZ in cyanobacterial phycoerythrin biosynthesis.

AB Pigment mutant strain FdR1 of the filamentous cyanobacterium *Fremyella diplosiphon* is characterized by constitutive synthesis of the **phycobiliprotein** phycoerythrin due to insertional inactivation of the rcaC regulatory gene by endogenous transposon Tn5469. Whereas the parental strain Fd33 harbors five genomic copies of Tn5469, cells of strain FdRI harbor six genomic copies of the element; the sixth copy in FdRI is localized to the rcaC gene. Electroporation of FdRI cells yielded secondary pigment mutant strains FdRIE1 and FdRIE4, which identically exhibited the FdRI phenotype with significantly reduced levels of phycoerythrin. In both FdRIE1 and FdRIE4, a seventh genomic copy of Tn5469 was localized to the cpeY gene of the sequenced but phenotypically uncharacterized cpeYZ gene set. This gene set is located downstream of the cpeBA operon which encodes the  $\alpha$  and  $\beta$  subunits of phycoerythrin. Complementation experiments correlated cpeYZ activity to the phenotype of strains FdRIE1 and FdRIE4. The predicted CpeY and CpeZ proteins share significant sequence identity with the products of homologous cpeY and cpeZ genes reported for *Pseudanabaena* sp. strain PCC 7409 and *Synechococcus* sp. strain WH 8020, both of which synthesize phycoerythrin. The CpeY and CpeZ proteins belong to a family of structurally related cyanobacterial proteins that includes the subunits of the CpcE/CpcF phycocyanin  $\alpha$ -subunit lyase of *Synechococcus* sp. strain PCC 7002 and the subunits of the PecE/PecF **phycoerythrocyanin**  $\alpha$ -subunit lyase of *Anabaena* sp. strain PCC 7120. Phycobilisomes isolated from mutant strains FdRIE1 and FdRIE4 contained equal amounts of chromophorylated  $\alpha$  and  $\beta$  subunits of phycoerythrin at 46% of the levels of the parental strain FdR1. These results suggest that the cpeYZ gene products function in phycoerythrin synthesis, possibly as a lyase involved in the attachment of phycoerythrobilin to the  $\alpha$  or  $\beta$  subunit.

ACCESSION NUMBER: 97052785 EMBASE

DOCUMENT NUMBER: 1997052785

TITLE: A role for cpeYZ in cyanobacterial phycoerythrin biosynthesis.

AUTHOR: Kahn K.; Mazel D.; Houmard J.; De Marsac N.T.; Schaefer M.R.

CORPORATE SOURCE: M.R. Schaefer, School of Biological Sciences, University of Missouri, 5100 Rockhill Rd., Kansas City, MO 64110, United States. mschaefer@cctr.umkc.edu

SOURCE: Journal of Bacteriology, (1997) 179/4 (998-1006).

Refs: 36

ISSN: 0021-9193 CODEN: JOBAAY

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

L6 ANSWER 35 OF 39 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI Candidate genes for the **phycoerythrocyanin**  $\alpha$ -subunit lyase. Biochemical analysis of pecE and pecF interposon mutants.

AB The rod substructures of the *Anabaena* sp. PCC 7120 phycobilisome contain the light harvesting proteins C-phycocyanin and **phycoerythrocyanin** (PEC). Even at low light intensities, PEC represents no more than 5% of the phycobilisome protein. The  $\beta$  subunits of both proteins carry thioether-linked phycocyanobilin (PCB) at  $\beta$ -Cys-82 and  $\beta$ -Cys-155; however, C-phycocyanin has PCB at  $\alpha$ -Cys-84 whereas PEC  $\alpha$ -subunit carries phycobiliviolin at this position. The *Anabaena* sp. PCC 7120 pec operon is made up of five genes. PecB and pecA encode the  $\beta$  and  $\alpha$  subunits of PEC, pecC encodes a linker polypeptide associated with PEC in the rod substructure, and pecE and pecF are genes of unknown function that show a high degree of homology to cpcE and cpcF,

that encode a C-phycocyanin .alpha. subunit PCB lyase (Fairchild, C. D., Zhao, J., Zhou, J., Colson, S. E., Bryant, D. A., and Glazer, A. N. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7017-7021). Insertional mutants in pecE and pecF, and an interposon mutant in which a portion of both pecE and pecF was deleted, were constructed. All three types of mutants grew 1.3 times slower than wild-type under limiting light conditions and showed a 20% reduction in the PCB content of whole cells relative to chlorophyll .alpha., Holo-PEC was missing from the phycobilisomes of all three types of mutants and the level of the PEC linker polypeptide was reduced relative to the wild-type. However, .apprx.30% of the wild-type level of the PEC .beta. subunit was present in all of these phycobilisomes. In contrast, the PEC .alpha. subunit was barely detectable in the pecE and pecF mutants, but was present in the pec EF deletion mutant as a PCB-adduct in a 1:1 ratio with the PEC .beta. subunit. The identity of this 'unnatural' adduct was confirmed by isolation of the subunit and amino-terminal sequencing. These biochemical results support the inference that pecE and pecF encode a PEC .alpha. subunit phycobiliviolin lyase, and, in conjunction with earlier findings, demonstrate that **phycobiliprotein** bilin lyases show high selectivity (rather than absolute specificity) for both the bilin and the polypeptide substrate.

ACCESSION NUMBER: 95162378 EMBASE  
DOCUMENT NUMBER: 1995162378  
TITLE: Candidate genes for the **phycoerythrocyanin** .alpha. subunit lyase. Biochemical analysis of pecE and pecF interposon mutants.  
AUTHOR: Jung L.J.; Chan C.F.; Glazer A.N.  
CORPORATE SOURCE: Stanley/Donner ASU, 229 Stanley Hall 3206, University of California, Berkeley, CA 94720-3206, United States  
SOURCE: Journal of Biological Chemistry, (1995) 270/21 (12877-12884).  
ISSN: 0021-9258 CODEN: JBCHA3  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 029 Clinical Biochemistry  
LANGUAGE: English  
SUMMARY LANGUAGE: English

L6 ANSWER 36 OF 39 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI The complete amino acid sequence of R-phycocyanin-I .alpha. and .beta. subunits from the red alga *Porphyridium cruentum*. Structural and phylogenetic relationships of the phycocyanins within the **phycobiliprotein** families.

AB We present here the complete primary structure of R-phycocyanin-I .alpha. and .beta. subunits from the red alga *Porphyridium cruentum*. The .alpha. chain is composed of 162 amino acid residues (18049 Da, calculated from sequence, including chromophore) and carries a phycocyanobilin pigment covalently linked to Cys84. The .beta. chain contains 172 amino acids (19344 Da, calculated from sequence, including chromophores) and carries a phycocyanobilin pigment covalently linked at Cys82 and a phycoerythrobin pigment at Cys153. A .gamma.-N-methyl asparagine residue was also characterised at position .beta.72 similar to other

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**phycobiliprotein** .beta. subunits. R-phycocyanin-I from *Porphyridium cruentum* shares high sequence identity with C-phycocyanins (69-83%), R-phycocyanins (66-70%) and in a less extent with phycoerythrocyanins (57-65%) from various sources. The presented phylogenetic trees are based on a comparison of all **phycobiliprotein** amino acid sequences known so far and confirm the clear affiliation of the R-phycocyanins in the phycocyanin family. In spite of their particular phycobilin pattern, they do not represent intermediate forms between the phycocyanin and the phycoerythrin family. **Phycoerythrocyanin**, a phycocyanin-related **phycobiliprotein** adapted to green light harvesting, is also shown to belong to the phycocyanin family. However, the phycoerythrocyanins diverge from phycocyanins in their different function and it is suggested that they

should be assigned to a separate group within the phycocyanin family.

ACCESSION NUMBER: 94116795 EMBASE  
DOCUMENT NUMBER: 1994116795  
TITLE: The complete amino acid sequence of R-phycocyanin-I .alpha. and .beta. subunits from the red alga *Porphyridium cruentum*. Structural and phylogenetic relationships of the phycocyanins within the **phycobiliprotein** families.  
AUTHOR: Ducret A.; Sidler W.; Frank G.; Zuber H.  
CORPORATE SOURCE: Inst. for Molec. Biology/Biophysics, Federal institute of Technology, CH-8093 Zurich, Switzerland  
SOURCE: European Journal of Biochemistry, (1994) 221/1 (563-580). ISSN: 0014-2956 CODEN: EJBCAI  
COUNTRY: Germany  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

L6 ANSWER 37 OF 39 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI Structure of the genes encoding the rod-core linker polypeptides of *Mastigocladus laminosus* phycobilisomes and functional aspects of the **phycobiliprotein**/linker-polypeptide interactions.

AB The 3' portion of the cpc operon in *Mastigocladus laminosus* encloses the genes 5'-cpcF-cpcG1-cpcG2-cpcG3 3'. The three cpcG genes encode different phycocyanin-associated rod-core linker polypeptides of the phycobilisomes with predicted 279, 247 and 254 amino acids in length. The gene products CpcG show a high similarity at their N-terminal domains (190 amino acids) and an overall identity of 47-53% to one another. Each of the three CpcG polypeptides is highly related to one of the four CpcG gene products of *Anabaena* sp. PCC 7120 (66-81% identity). It is suggested that these pairs of rod-core linker polypeptides mediate the same specific type of phycocyanin .fwdarw. allophycocyanin interaction in the similar phycobilisomes of *M. laminosus* and *Anabaena* sp. PCC 7120. The similarity of the CpcG1, CpcG2 and CpcG3 polypeptides to the single CpcG rod-core linker polypeptide of *Synechococcus* sp. PCC 7002 (36-41% identity) is lower. The rod-core linker polypeptides are more distantly related to the rod linker polypeptides associated with phycocyanin or phycoerythrin. However, six conserved domains were identified within the N-terminal 190 amino acids of these linker proteins, which bear similar amino acid sequences, including highly conserved basic amino acids. A similar amino acid sequence but with conserved acidic amino acids can be found in the .beta. subunits of phycocyanin, phycoerythrin and **phycoerythrocyanin**, which is protruding into the central cavity of the **phycobiliprotein** hexamers. It is suggested that these domains are sites of **phycobiliprotein**-hexamer/rod and rod-core linker interactions.

ACCESSION NUMBER: 92141649 EMBASE  
DOCUMENT NUMBER: 1992141649  
TITLE: Structure of the genes encoding the rod-core linker polypeptides of *Mastigocladus laminosus* phycobilisomes and functional aspects of the **phycobiliprotein**/linker-polypeptide interactions.  
AUTHOR: Glauser M.; Stirewalt V.L.; Bryant D.A.; Sidler W.; Zuber H.  
CORPORATE SOURCE: Inst. fur Molekularbiol./Biophysik, ETH-Honggerberg-HPM, CH-8093 Zurich, Switzerland  
SOURCE: European Journal of Biochemistry, (1992) 205/3 (927-937). ISSN: 0014-2956 CODEN: EJBCAI  
COUNTRY: Germany  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

L6 ANSWER 38 OF 39 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
 TI Refined three-dimensional structure of **phycoerythrocyanin** from the cyanobacterium *Mastigocladus laminosus* at 2.7 .ANG..  
 AB The structure of the **phycobiliprotein phycoerythrocyanin** from the thermophilic cyanobacterium *Mastigocladus laminosus* has been determined at 2.7 .ANG. resolution by X-ray diffraction methods on the basis of the molecular model of C-phycoerythrocyanin from the same organism. Hexagonal **phycoerythrocyanin** crystals of space group P63 with cell constants a=b=156.86 .ANG., c=40.39 .ANG., .alpha.=.beta.=90.degree., .gamma.=120.degree. are almost isomorphous to C-phycoerythrocyanin crystals. The crystal structure has been refined by energy-restrained crystallographic refinement and model building. The conventional crystallographic R-factor of the final model was 19.2% with data to 2.7 .ANG. resolution. In **phycoerythrocyanin**, the three (.alpha..beta.)-subunits are arranged around a 3-fold symmetry axis, as in C-phycoerythrocyanin. The two structures are very similar. After superposition, the 162 C(.alpha.) atoms of the .alpha.-subunit have a mean difference of 0.71 .ANG. and the 171 C(.alpha.) atoms of the .beta.-subunit differ by 0.51 .ANG.. The stereochemistry of the chiral atoms in the phycobiliviolin chromophore A84 is C((31))-R, C((4))-S. The configuration of the chromophore is C((10))-Z, C((15))-Z and the conformation C((5))-anti, C((9))-syn and C((14))-anti like the phycocyanobilin chromophores in **phycoerythrocyanin** and C-phycoerythrocyanin.

ACCESSION NUMBER: 90086271 EMBASE  
 DOCUMENT NUMBER: 1990086271  
 TITLE: Refined three-dimensional structure of **phycoerythrocyanin** from the cyanobacterium *Mastigocladus laminosus* at 2.7 .ANG..  
 AUTHOR: Duerring M.; Huber R.; Bode W.; Ruembeli R.; Zuber H.  
 CORPORATE SOURCE: Max-Planck Institut fur Biochemie, D-8033 Martinsried, Germany  
 SOURCE: Journal of Molecular Biology, (1990) 211/3 (633-644).  
 ISSN: 0022-2836 CODEN: JMOBAK  
 COUNTRY: United Kingdom  
 DOCUMENT TYPE: Journal; Article  
 FILE SEGMENT: 004 Microbiology  
 LANGUAGE: English  
 SUMMARY LANGUAGE: English

L6 ANSWER 39 OF 39 WPIDS (C) 2003 THOMSON DERWENT  
 TI Analyzing soluble analyte concentration in sample, useful for diagnosing disease, by performing competition assay using formed bodies to which are attached at least one analyte, unlabeled and labeled ligands of analyte.  
 AN 2003-040599 [03] WPIDS  
 AB WO 200277645 A UPAB: 20030113  
 NOVELTY - Analyzing (M1) concentration of soluble analyte (I) comprising utilizing formed bodies (II) to which analyte is bound, performing competition assay in which unlabeled and labeled ligands (UL,LL) compete for binding to (II)-bound analyte and (I), incubating control samples containing (II) and varying known concentrations of LL with no UL, analyzing test and control samples to obtain detectable signal from LL, and using the data to determine concentration of (I), is new.

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DETAILED DESCRIPTION - Analyzing (M1) the concentration or amount of soluble analyte (I) in a sample, involves incubating test samples containing an unknown concentration of (I), a predetermined amount of formed bodies to which are attached at least one analyte, varying known concentrations of unlabeled ligand (UL) that binds to the analyte, and a known concentration of the ligand labeled with a detectable marker, where LL and the UL compete for binding to the formed body-bound analyte and (I); incubating several control samples which contains predetermined amount of the formed bodies and varying known concentrations of LL with no UL to permit binding to reach equilibrium, where the formed bodies are between 1-100% coated with LL; analyzing test samples and control samples

in an instrument that measures detectable signal produced from LLs bound to the bound analytes on the formed bodies; identifying the intersection of a curve formed by plotting the signal against the concentration of LL in the control samples, and a second curve formed by plotting the signal against the concentration of total labeled and UL in the test samples; determining the concentration of UL that bound to (I) in the test samples by evaluating the difference D between the concentration that corresponds with the intersection and the constant LL concentration in the test samples; and determining the concentration of (I) in the sample by determining the product of the value D and the binding stoichiometry of the ligand to (I).

INDEPENDENT CLAIMS are also included for:

(1) a computer program that identifies and analyzes the amount of (I) in a sample by (M1);

(2) an analysis instrument that comprises an integrated computer program that identifies and analyzes the amount of (I) in a sample by implementing (M1); and

(3) a kit for performing a method of diagnosing a disease characterized by an altered level of (I) in the blood of a mammal, comprises components selected from ligands, detectable markers for labeling a ligand, formed bodies, suitable vessels for containing samples, suitable controls or tables of normal values of (I), instructions for performing the method and preparing the controls, suitable diluents and buffers for the samples, indicator charts for signal comparisons, disposable gloves, decontamination instructions, applicator sticks or containers, and/or sample preparator cups.

USE - (M1) is useful for analyzing concentration or amount of soluble analyte in a sample which is purified of any particles other than the analyte, and contains formed bodies having the analytes bound to it, and (I) shed from the formed bodies. The method is useful for analyzing concentration or amount of soluble analyte which is a proteinaceous or chemical composition which can be naturally or covalently bound to a formed body, e.g. cell surface receptor, preferably an antigenic receptor anchored to a white blood cell by a glycosyl-phosphatidylinositol linkage. (M1) is useful for analyzing the concentration or amount of soluble receptor from a sample which comprises formed bodies having the receptors bound to them, and the soluble receptors shed from the formed bodies, where the concentration of soluble receptors is calculated according to the formula  $D \times \text{multiply binding valence of the ligand}$ . (M1) is useful for diagnosing a disease characterized by an altered level of (I) in the blood of a mammal which involves analyzing the amount of (I) by performing (M1), comparing the concentration of (I) from the mammal's samples with known normal concentrations of (I) in samples of healthy mammalian blood, where a difference between the concentration of (I) in the mammal's blood sample and the normal concentration is indicative of disease. Preferably the method is useful for diagnosing a disease characterized by an altered level of soluble receptors shed by target cells having receptors bound to the cell surface into the blood of a mammal (all claimed). The method has medical applications such as diagnosis of diseases characterized by presence of certain analytes in a biological sample, or the evaluation of commercial or industrial samples containing analytes. The method is also useful for monitoring the efficacy of treatment of various diseases characterized by shedding of bound receptors from target cells e.g. blood cells and other diseases in which the receptors or analytes are not naturally bound but are soluble. The method is useful for analyzing soluble and neutrophil-bound CD16b antigen, and for quantifying many other soluble and cell bound receptors, e.g. the CD100 receptor shed in the context of spinal cord injury. The method can be also be used to determine only (I), such as for thyroid factor T4 or soluble prostate surface antigen. The method is useful for distinguishing between the normal and diseased form of the prion protein, for quantifying non-biological analytes, e.g. for particular analytes in water or other fluids which the proteins are soluble.

ADVANTAGE - (M1) is simple, and enables rapid flow cytometric

analysis of samples. The method requires no pure or partially pure antigen, and lacks any requirement for an extraneous solid support for any component of the reaction. The elimination of these requirements allows the assays to be more easily performed in samples which contain preexisting formed bodies and receptors. The method is both simple and inexpensive requiring only a supply of purified unlabeled ligand and suitable fluorescent LL against the same analyte. The method does not involve lengthy incubation times or precautions regarding the use and disposal of radioactive material.

Dwg.1A/3

ACCESSION NUMBER: 2003-040599 [03] WPIDS  
 DOC. NO. NON-CPI: N2003-031888  
 DOC. NO. CPI: C2003-009585  
 TITLE: Analyzing soluble analyte concentration in sample, useful for diagnosing disease, by performing competition assay using formed bodies to which are attached at least one analyte, unlabeled and labeled ligands of analyte.  
 DERWENT CLASS: A89 B04 D16 S03  
 INVENTOR(S): SIIMAN, O  
 PATENT ASSIGNEE(S): (SIIM-I) SIIMAN O; (COUS) COULTER INT CORP  
 COUNTRY COUNT: 21  
 PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002077645	A2	20021003	(200303)*	EN	45
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR					
W: JP					
US 2002142289	A1	20021003	(200303)		

#### APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002077645	A2	WO 2002-US3176	20020102
US 2002142289	A1	US 2001-768127	20010123

PRIORITY APPLN. INFO: US 2001-768127 20010123

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=> s 15 and 18  
 L9 9 L5 AND L8

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L9 ANSWER 1 OF 9 MEDLINE  
 TI Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo-alpha subunit in a heterologous host.  
 AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (Synechocystis sp. PCC6803) was reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phycocyanin alpha subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant

**E. coli** used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin addition took place in a similarly engineered **E. coli** strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in **phycobiliprotein** biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001504133 MEDLINE  
 DOCUMENT NUMBER: 21438034 PubMed ID: 11553806  
 TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo-alpha subunit in a heterologous host.  
 AUTHOR: Tooley A J; Cai Y A; Glazer A N  
 CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, 142 LSA no. 3200, Berkeley, CA 94720-3200, USA.  
 SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Sep 11) 98 (19) 10560-5. Journal code: 7505876. ISSN: 0027-8424.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200111  
 ENTRY DATE: Entered STN: 20010913  
 Last Updated on STN: 20011105  
 Entered Medline: 20011101

L9 ANSWER 2 OF 9 MEDLINE

TI Expression of Escherichia coli phosphoenolpyruvate carboxylase in a cyanobacterium. Functional complementation of Synechococcus PCC 7942 ppc.  
 AB The gene (ppc) coding for phosphoenolpyruvate carboxylase (PEPCase) in the cyanobacterium Synechococcus PCC 7942 has been inactivated via insertional mutagenesis while being functionally complemented by Escherichia coli ppc. Cyanobacterial cells functionally complemented by **E. coli** ppc showed decreased PEPCase activity in crude cell lysates and detergent-permeabilized whole cell assays. Decreased rates of growth, reduced levels of chlorophyll a, and decreased photosynthetic O<sub>2</sub> evolution capacity per cell when compared to wild-type cyanobacterial cells were also observed. **Phycobiliprotein** levels were not affected. The results are discussed in terms of the impact of reduced PEPCase activity on cyanobacterial cell metabolism and the regulatory properties of the **E. coli** gene product.

ACCESSION NUMBER: 94105286 MEDLINE  
 DOCUMENT NUMBER: 94105286 PubMed ID: 8278492  
 TITLE: Expression of Escherichia coli phosphoenolpyruvate carboxylase in a cyanobacterium. Functional complementation of Synechococcus PCC 7942 ppc.  
 AUTHOR: Luinenburg I; Coleman J R  
 CORPORATE SOURCE: Department of Botany, University of Toronto, Ontario, Canada.  
 SOURCE: PLANT PHYSIOLOGY, (1993 Jan) 101 (1) 121-6. Journal code: 0401224. ISSN: 0032-0889.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199402  
 ENTRY DATE: Entered STN: 19940218  
 Last Updated on STN: 19940218  
 Entered Medline: 19940208

L9 ANSWER 3 OF 9 MEDLINE

TI Isolation and characterization of the genes encoding allophycocyanin subunits and two linker proteins from *Synechocystis* 6714.

AB Genes encoding the phycobilisome core subunits allophycocyanin alpha and beta and a small core linker protein in *Synechocystis* sp. strain PCC 6714 were cloned and sequenced. These genes form an operon, *apcABC*, with a single transcription start site and two possible termination sites, one following *apcB* and the other following *apcC*. The promoter region, like those of the *apcABC* operons of other cyanobacteria, does not resemble the consensus promoter sequences of *Escherichia coli*. However, the *apcABC* promoters identified in four strains of cyanobacteria have conserved sequences centered at -50 and -10 with respect to the start of transcription. The *apcE* gene, encoding the protein that links the phycobilisome core to the thylakoid membrane, was also cloned from *Synechocystis* 6714 and sequenced. It is unlinked to the *apcABC* operon. As in other *Synechocystis* strains, the LCM polypeptide encoded by the *apcE* gene contains three repeats of the basic **phycobiliprotein** linker domain. The *apcE* gene promoter sequence bears little resemblance to either the *E. coli* consensus or the *apcABC* promoter region, but it is similar to the corresponding regions of other cyanobacterial *apcE* genes. In these cases, there are conserved sequences centered at -40 and -10 with respect to the transcription start site. These conserved promoter elements from the *apcABC* and *apcE* genes were also identified in the corresponding 5'-flanking regions of eleven transcript starts for *cpc* genes encoding phycocyanin subunits in cyanobacteria and algal chloroplasts. These results suggest that a factor yet to be described participates in transcription of **phycobiliprotein** genes.

ACCESSION NUMBER: 93222481 MEDLINE  
DOCUMENT NUMBER: 93222481 PubMed ID: 8467079  
TITLE: Isolation and characterization of the genes encoding allophycocyanin subunits and two linker proteins from *Synechocystis* 6714.  
AUTHOR: DiMagno L; Haselkorn R  
CORPORATE SOURCE: Department of Chemistry and Molecular Genetics, University of Chicago, IL 60637.  
CONTRACT NUMBER: GM 08282 (NIGMS)  
SOURCE: PLANT MOLECULAR BIOLOGY, (1993 Mar) 21 (5) 835-45.  
Journal code: 9106343. ISSN: 0167-4412.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-X06084; GENBANK-Z11906  
ENTRY MONTH: 199305  
ENTRY DATE: Entered STN: 19930521  
Last Updated on STN: 19930521  
Entered Medline: 19930511

L9 ANSWER 4 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

TI Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo-alpha subunit in a heterologous host.

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid *trp-lac* (*trc*) promoter. Genes for the apoprotein (C-phycocyanin alpha subunit; *cpcA*) and the heterodimeric lyase (*cpcE* and *cpcF*) that catalyzes chromophore attachment were expressed from the *trc* promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA.

No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks *cpcE* and *cpcF*. This approach should permit incisive analysis of many remaining questions in **phycobiliprotein** biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001:482056 BIOSIS  
DOCUMENT NUMBER: PREV200100482056  
TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo-alpha subunit in a heterologous host.  
AUTHOR(S): Tooley, Aaron J.; Cai, Yuping A.; Glazer, Alexander N. (1)  
CORPORATE SOURCE: (1) Natural Reserve System, University of California System, 1111 Franklin Street, 6th Floor, Oakland, CA, 94607-5200: alexander.glazer@ucop.edu USA  
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (September 11, 2001) Vol. 98, No. 19, pp. 10560-10565. print.  
ISSN: 0027-8424.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
SUMMARY LANGUAGE: English

L9 ANSWER 5 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
TI Isolation and characterization of the genes encoding allophycocyanin subunits and two linker proteins from *Synechocystis* 6714.  
AB Genes encoding the phycobilisome core subunits allophycocyanin alpha and beta and a small core linker protein in *Synechocystis* sp. strain PCC 6714 were cloned and sequenced. These genes form an operon, *apcABC*, with a single transcription start site and two possible termination sites, one following *apcB* and the other following *apcC*. The promoter region, like those of the *apcABC* operons of other cyanobacteria, does not resemble the consensus promoter sequences of *Escherichia coli*. However, the *apcABC* promoters identified in four strains of cyanobacteria have conserved sequences centered at -50 and -10 with respect to the start of transcription. The *apcE* gene, encoding the protein that links the phycobilisome core to the thylakoid membrane, was also cloned from *Synechocystis* 6714 and sequenced. It is unlinked to the *apcABC* operon. As in other *Synechocystis* strains, the L-CM polypeptide encoded by the *apcE* gene contains three repeats of the basic **phycobiliprotein** linker domain. The *apcE* gene promoter sequence bears little resemblance to either the *E. coli* consensus or the *apcABC* promoter region, but it is similar to the corresponding regions of other cyanobacterial *apcE* genes. In these cases, there are conserved sequences centered at -40 and -10 with respect to the transcription start site. These conserved promoter elements from the *apcABC* and *apcE* genes were also identified in the corresponding 5'-flanking regions of eleven transcript starts for *cpc* genes encoding phycocyanin subunits in cyanobacteria and algal chloroplasts. These results suggest that a factor yet to be described participates in transcription of **phycobiliprotein** genes.

ACCESSION NUMBER: 1993:319341 BIOSIS  
DOCUMENT NUMBER: PREV199396027691  
TITLE: Isolation and characterization of the genes encoding allophycocyanin subunits and two linker proteins from *Synechocystis* 6714.  
AUTHOR(S): Dimagno, Lisa; Haselkorn, Robert (1)  
CORPORATE SOURCE: (1) Dep. Chem., Univ. Chicago, 920 East 58 St., Chicago, IL 60637 USA  
SOURCE: Plant Molecular Biology, (1993) Vol. 21, No. 5, pp. 835-845.  
ISSN: 0167-4412.  
DOCUMENT TYPE: Article  
LANGUAGE: English

L9 ANSWER 6 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

TI Expression of Escherichia coli phosphoenolpyruvate carboxylase in a cyanobacterium: Functional complementation of Synechococcus PCC 7942 ppc.  
AB The gene (ppc) coding for phosphoenolpyruvate carboxylase (PEPCase) in the cyanobacterium Synechococcus PCC 7942 has been inactivated via insertional mutagenesis while being functionally complemented by Escherichia coli ppc. Cyanobacterial cells functionally complemented by *E. coli* ppc showed decreased PEPCase activity in crude cell lysates and detergent-permeabilized whole cell assays: Decreased rates of growth, reduced levels of chlorophyll a, and decreased photosynthetic O-2 evolution capacity per cell when compared to wild-type cyanobacterial cells were also observed. **Phycobiliprotein** levels were not affected. The results are discussed in terms of the impact of reduced PEPCase activity on cyanobacterial cell metabolism and the regulatory properties of the *E. coli* gene product.

ACCESSION NUMBER: 1993:164233 BIOSIS  
DOCUMENT NUMBER: PREV199395085283  
TITLE: Expression of Escherichia coli phosphoenolpyruvate carboxylase in a cyanobacterium: Functional complementation of Synechococcus PCC 7942 ppc.  
AUTHOR(S): Luinenburg, Irene; Coleman, John R. (1)  
CORPORATE SOURCE: (1) Dep. Bot., Univ. Toronto, Toronto, ON, Can. M5S 3B2  
SOURCE: Plant Physiology (Rockville), (1993) Vol. 101, No. 1, pp. 121-126.  
ISSN: 0032-0889.  
DOCUMENT TYPE: Article  
LANGUAGE: English

L9 ANSWER 7 OF 9 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI Biosynthesis of the cyanobacterial light-harvesting polypeptide phycoerythrocyanin holo-.alpha. subunit in a heterologous host.  
AB The entire pathway for the biosynthesis of the phycobiliviolin-bearing His-tagged holo-.alpha. subunit of the cyanobacterial photosynthetic accessory protein phycoerythrocyanin was reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes required for the conversion of heme to 3Z-phycoerythrocyanin, a precursor of phycobiliviolin (namely, heme oxygenase 1 and 3Z-phycoerythrocyanin:ferredoxin oxidoreductase), were expressed from a plasmid under the control of the hybrid trp-lac (trc) promoter. Genes for the apo-phycoerythrocyanin .alpha. subunit (pecA) and the heterodimeric lyase/isomerase (pecE and pecF), which catalyzes both the covalent attachment of phycoerythrocyanin and its concurrent isomerization to phycobiliviolin, were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used endogenous heme to produce holo-PecA with absorbance and fluorescence properties similar to those of the same protein produced in cyanobacteria. About two-thirds of the apo-PecA was converted to holo-PecA. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks pecE and pecF. By using immobilized metal affinity chromatography, both apo-PecA and holo-PecA were isolated as ternary complexes with PecE and PecF. The identities of all three components in the ternary complexes were established unambiguously by protein and tryptic peptide analyses performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

ACCESSION NUMBER: 2002295599 EMBASE  
TITLE: Biosynthesis of the cyanobacterial light-harvesting polypeptide phycoerythrocyanin holo-.alpha. subunit in a heterologous host.  
AUTHOR: Tooley A.J.; Glazer A.N.  
CORPORATE SOURCE: A.N. Glazer, Natural Reserve System, University of California, 1111 Franklin Street, Oakland, CA 94607-5200, United States. glazer@uclink4.berkeley.edu  
SOURCE: Journal of Bacteriology, (2002) 184/17 (4666-4671).  
Refs: 22  
ISSN: 0021-9193 CODEN: JOBAAY  
COUNTRY: United States

DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

L9 ANSWER 8 OF 9 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI Biosynthesis of a fluorescent cyanobacterial C-phyocyanin holo-.alpha. subunit in a heterologous host.

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phyocyanobilin, namely, heme oxygenase 1 and 3Z-phyocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phyocyanin .alpha. subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in **phycobiliprotein** biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001329835 EMBASE  
TITLE: Biosynthesis of a fluorescent cyanobacterial C-phyocyanin holo-.alpha. subunit in a heterologous host.  
AUTHOR: Tooley A.J.; Cai Y.A.; Glazer A.N.  
CORPORATE SOURCE: A.N. Glazer, Natural Reserve System, University of California System, 1111 Franklin Street, Oakland, CA 94607-5200, United States. alexander.glazer@ucop.edu  
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (11 Sep 2001) 98/19 (10560-10565).  
Refs: 30  
ISSN: 0027-8424 CODEN: PNASA6  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

L9 ANSWER 9 OF 9 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI Expression of **phycobiliprotein** genes in *Escherichia coli*.

AB The cpcA and cpcB genes, which encode the .alpha.- and .beta.-subunit apoproteins of *Synechococcus* 7002 phycocyanin (PC), respectively, are efficiently expressed in *Escherichia coli*. Studies performed with a series of deletions produced from the parental plasmid pAQPR1 suggest that transcription of these genes in *E. coli* is initiated from a promoter located not more than 384 bp upstream from the translation start of cpcB. An examination of the nucleotide sequence of this region reveals a consensus *E. coli* promoter centered approx. 374 bp upstream from the cpcB translation start. The cyanelle-encoded allophycocyanin .alpha.- and .beta.-subunit apoprotein genes (apcA and apcB) of *Cyanophora paradoxa* are also expressed in *E. coli*.

ACCESSION NUMBER: 86005229 EMBASE  
DOCUMENT NUMBER: 1986005229  
TITLE: Expression of **phycobiliprotein** genes in *Escherichia coli*.

AUTHOR: Bryant D.A.; Dubbs J.M.; Fields P.I.; et al.  
CORPORATE SOURCE: Department of Molecular and Cell Biology, The Pennsylvania  
State University, University Park, PA 16802, United States  
SOURCE: FEMS Microbiology Letters, (1985) 29/3 (343-349).  
CODEN: FMLED7  
COUNTRY: Netherlands  
DOCUMENT TYPE: Journal  
FILE SEGMENT: 004 Microbiology  
022 Human Genetics  
LANGUAGE: English

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NEWS	35	Dec 17	PCTFULL now covers WP/PCT Applications from 1978 to date
NEWS	36	Dec 17	TOXCENTER enhanced with additional content
NEWS	37	Dec 17	Adis Clinical Trials Insight now available on STN
NEWS	38	Dec 30	ISMEC no longer available
NEWS	39	Jan 21	NUTRACEUT offering one free connect hour in February 2003
NEWS	40	Jan 21	PHARMAML offering one free connect hour in February 2003
NEWS	41	Jan 29	Simultaneous left and right truncation added to COMPENDEX, ENERGY, INSPEC
NEWS	42	Feb 13	CANCERLIT is no longer being updated
NEWS	43	Feb 24	METADEX enhancements
NEWS	44	Feb 24	PCTGEN now available on STN
NEWS	45	Feb 24	TEMA now available on STN

NEWS 46 Feb 26 NTIS now allows simultaneous left and right truncation  
 NEWS 47 Feb 26 PCTFULL now contains images  
 NEWS 48 Mar 04 SDI PACKAGE for monthly delivery of multifile SDI results  
 NEWS 49 Mar 19 APOLLIT offering free connect time in April 2003  
 NEWS 50 Mar 20 EVENTLINE will be removed from STN  
 NEWS 51 Mar 24 PATDPAFULL now available on STN  
 NEWS 52 Mar 24 Additional information for trade-named substances without  
 structures available in REGISTRY  
 NEWS 53 Mar 24 Indexing from 1957 to 1966 added to records in CA/CAPLUS

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=> s apo-enzyme

L1 565 APO-ENZYME

=> s holo-enzyme

L2 455 HOLO-ENZYME

=> s l2 and l1

L3 81 L2 AND L1

=> s phycobiliprotein

L4 1404 PHYCOBILIPROTEIN

=> s l4 and l2

L5 0 L4 AND L2

=> s l4 and holo

L6 11 L4 AND HOLO

=> d l6 ti abs ibib tot

L6 ANSWER 1 OF 11 MEDLINE

TI Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo**  
-alpha subunit in a heterologous host.

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phycocyanin alpha subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce **holo**-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to **holo**-CpcA. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in **phycobiliprotein** biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001504133 MEDLINE

DOCUMENT NUMBER: 21438034 PubMed ID: 11553806

TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo**-alpha subunit in a heterologous host.

AUTHOR: Tooley A J; Cai Y A; Glazer A N

CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, 142 LSA no. 3200, Berkeley, CA 94720-3200, USA:

SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Sep 11) 98 (19) 10560-5. Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

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ENTRY MONTH: 200111  
ENTRY DATE: Entered STN: 20010913  
Last Updated on STN: 20011105  
Entered Medline: 20011101

L6 ANSWER 2 OF 11 MEDLINE

TI Candidate genes for the phycoerythrocyanin alpha subunit lyase.

Biochemical analysis of pecE and pecF interposon mutants.

AB The rod substructures of the Anabaena sp. PCC 7120 phycobilisome contain the light harvesting proteins C-phycocyanin and phycoerythrocyanin (PEC). Even at low light intensities, PEC represents no more than 5% of the phycobilisome protein. The beta subunits of both proteins carry thioether-linked phycocyanobilin (PCB) at beta-Cys-82 and beta-Cys-155; however, C-phycocyanin has PCB at alpha-Cys-84 whereas PEC alpha subunit carries phycobiliviolin at this position. The Anabaena sp. PCC 7120 pec operon is made up of five genes. PecB and pecA encode the beta and alpha subunits of PEC, pecC encodes a linker polypeptide associated with PEC in the rod substructure, and pecE and pecF are genes of unknown function that show a high degree of homology to cpcE and cpcF, that encode a C-phycocyanin alpha subunit PCB lyase (Fairchild, C. D., Zhao, J., Zhou, J., Colson, S. E., Bryant, D. A., and Glazer, A. N. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 7017-7021). Insertional mutants in pecE and pecF, and an interposon mutant in which a portion of both pecE and pecF was deleted, were constructed. All three types of mutants grew 1.3 times slower than wild-type under limiting light conditions and showed a 20% reduction in the PCB content of whole cells relative to chlorophyll alpha. Holo-PEC was missing from the phycobilisomes of all three types of mutants and the level of the PEC linker polypeptide was reduced relative to the wild-type. However, approximately 30% of the wild-type level of the PEC beta subunit was present in all of these phycobilisomes. In contrast, the PEC alpha subunit was barely detectable in the pecE and pecF mutants, but was present in the pecEF deletion mutant as a PCB-adduct in a 1:1 ratio with the PEC beta subunit. The identity of this "unnatural" adduct was confirmed by isolation of the subunit and amino-terminal sequencing. These biochemical results support the inference that pecE and pecF encode a PEC alpha subunit phycobiliviolin lyase, and, in conjunction with earlier findings, demonstrate that **phycobiliprotein** bilin lyases show high selectivity (rather than absolute specificity) for both the bilin and the polypeptide substrate.

ACCESSION NUMBER: 95279433 MEDLINE  
DOCUMENT NUMBER: 95279433 PubMed ID: 7759546  
TITLE: Candidate genes for the phycoerythrocyanin alpha subunit lyase. Biochemical analysis of pecE and pecF interposon mutants.  
AUTHOR: Jung L J; Chan C F; Glazer A N  
CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, Berkeley 94720, USA.  
CONTRACT NUMBER: GM28994 (NIGMS)  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 May 26) 270 (21) 12877-84.  
Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199506  
ENTRY DATE: Entered STN: 19950707  
Last Updated on STN: 19950707  
Entered Medline: 19950628

L6 ANSWER 3 OF 11 USPATFULL

TI Engineering of living cells for the expression of **holo-phycobiliprotein-based constructs**

AB Recombinant cells which express a fluorescent holo-

**phycobiliprotein** fusion protein and methods of use are described. The cells comprises a bilin, a recombinant bilin reductase, an apo-**phycobiliprotein** fusion protein precursor of the fusion protein comprising a corresponding apo-**phycobiliprotein** domain, and a recombinant **phycobiliprotein** domain-bilin lyase, which components react to form the **holo-phycobiliprotein** fusion protein. Also described are **holo-phycobiliprotein** based transcription reporter cells and assays, which cells conditionally express a heterologous-to-the-cell, fluorescent, first **holo-phycobiliprotein** domain.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2003:37640 USPATFULL

TITLE: Engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs

INVENTOR(S): Glazer, Alexander N., Berkeley, CA, UNITED STATES  
Tooley, Aaron J., Berkeley, CA, UNITED STATES  
Cai, Yuping, Carmel, IN, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003027285	A1	20030206
APPLICATION INFO.:	US 2001-919486	A1	20010731 (9)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	RICHARD ARON OSMAN, SCIENCE AND TECHNOLOGY LAW GROUP, 75 DENISE DRIVE, HILLSBOROUGH, CA, 94010		
NUMBER OF CLAIMS:	24		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	3 Drawing Page(s)		
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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TI Biosynthesis of the cyanobacterial light-harvesting polypeptide phycoerythrocyanin **holo**-.alpha. subunit in a heterologous host.

AB The entire pathway for the biosynthesis of the phycobiliviolin-bearing His-tagged **holo**-.alpha. subunit of the cyanobacterial photosynthetic accessory protein phycoerythrocyanin was reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes required for the conversion of heme to 3Z-phycoerythrocyanin, a precursor of phycobiliviolin (namely, heme oxygenase 1 and 3Z-phycoerythrocyanin:ferredoxin oxidoreductase), were expressed from a plasmid under the control of the hybrid trp-lac (trc) promoter. Genes for the apo-phycoerythrocyanin .alpha. subunit (pecA) and the heterodimeric lyase/isomerase (pecE and pecF), which catalyzes both the covalent attachment of phycoerythrocyanin and its concurrent isomerization to phycobiliviolin, were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used endogenous heme to produce **holo**-PecA with absorbance and fluorescence properties similar to those of the same protein produced in cyanobacteria. About two-thirds of the apo-PecA was converted to **holo**-PecA. No significant bilin addition took place in a similarly engineered E. coli strain that lacks pecE and pecF. By using immobilized metal affinity chromatography, both apo-PecA and **holo**-PecA were isolated as ternary complexes with PecE and PecF. The identities of all three components in the ternary complexes were established unambiguously by protein and tryptic peptide analyses performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

ACCESSION NUMBER: 2002295599 EMBASE

TITLE: Biosynthesis of the cyanobacterial light-harvesting polypeptide phycoerythrocyanin **holo**-.alpha. subunit in a heterologous host.

AUTHOR: Tooley A.J.; Glazer A.N.

CORPORATE SOURCE: A.N. Glazer, Natural Reserve System, University of California, 1111 Franklin Street, Oakland, CA 94607-5200, United States. glazer@uclink4.berkeley.edu  
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DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

L6 ANSWER 5 OF 11 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
TI Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo**-.alpha. subunit in a heterologous host.  
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TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo**-.alpha. subunit in a heterologous host.  
AUTHOR: Tooley A.J.; Cai Y.A.; Glazer A.N.  
CORPORATE SOURCE: A.N. Glazer, Natural Reserve System, University of California System, 1111 Franklin Street, Oakland, CA 94607-5200, United States. alexander.glazer@ucop.edu  
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (11 Sep 2001) 98/19 (10560-10565).  
Refs: 30  
ISSN: 0027-8424 CODEN: PNASA6  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

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L6 ANSWER 6 OF 11 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
TI Candidate genes for the phycoerythrocyanin .alpha. subunit lyase. Biochemical analysis of pecE and pecF interposon mutants.  
AB The rod substructures of the *Anabaena* sp. PCC 7120 phycobilisome contain the light harvesting proteins C-phycocyanin and phycoerythrocyanin (PEC). Even at low light intensities, PEC represents no more than 5% of the phycobilisome protein. The .beta. subunits of both proteins carry thioether-linked phycocyanobilin (PCB) at .beta.-Cys-82 and .beta.-Cys-155; however, C-phycocyanin has PCB at .alpha.-Cys-84 whereas PEC .alpha. subunit carries phycobiliviolin at this position. The *Anabaena* sp. PCC 7120 pec operon is made up of five genes. PecB and pecA encode the .beta. and .alpha. subunits of PEC, pecC encodes a linker polypeptide

associated with PEC in the rod substructure, and pecE and pecF are genes of unknown function that show a high degree of homology to cpcE and cpcF, that encode a C-phytyocyanin .alpha. subunit PCB lyase (Fairchild, C. D., Zhao, J., Zhou, J., Colson, S. E., Bryant, D. A., and Glazer, A. N. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7017-7021). Insertional mutants in pecE and pecF, and an interposon mutant in which a portion of both pecE and pecF was deleted, were constructed. All three types of mutants grew 1.3 times slower than wild-type under limiting light conditions and showed a 20% reduction in the PCB content of whole cells relative to chlorophyll .alpha., Holo-PEC was missing from the phycobilisomes of all three types of mutants and the level of the PEC linker polypeptide was reduced relative to the wild-type. However, .apprx.30% of the wild-type level of the PEC .beta. subunit was present in all of these phycobilisomes. In contrast, the PEC .alpha. subunit was barely detectable in the pecE and pecF mutants, but was present in the pec EF deletion mutant as a PCB-adduct in a 1:1 ratio with the PEC .beta. subunit. The identity of this 'unnatural' adduct was confirmed by isolation of the subunit and amino-terminal sequencing. These biochemical results support the inference that pecE and pecF encode a PEC .alpha. subunit phycobiliviolin lyase, and, in conjunction with earlier findings, demonstrate that **phycobiliprotein** bilin lyases show high selectivity (rather than absolute specificity) for both the bilin and the polypeptide substrate.

ACCESSION NUMBER: 95162378 EMBASE  
DOCUMENT NUMBER: 1995162378  
TITLE: Candidate genes for the phycoerythrocyanin .alpha. subunit lyase. Biochemical analysis of pecE and pecF interposon mutants.  
AUTHOR: Jung L.J.; Chan C.F.; Glazer A.N.  
CORPORATE SOURCE: Stanley/Donner ASU, 229 Stanley Hall 3206, University of California, Berkeley, CA 94720-3206, United States  
SOURCE: Journal of Biological Chemistry, (1995) 270/21 (12877-12884).  
ISSN: 0021-9258 CODEN: JBCHA3  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 029 Clinical Biochemistry  
LANGUAGE: English  
SUMMARY LANGUAGE: English

L6 ANSWER 7 OF 11 HCAPLUS COPYRIGHT 2003 ACS

TI Engineering of living cells for the expression of **holo-phycobiliprotein-based constructs**

AB Recombinant cells which express a fluorescent **holo-phycobiliprotein** fusion protein and methods of use are described. The cells comprises a bilin, a recombinant bilin reductase, an apo-**phycobiliprotein** fusion protein precursor of the fusion protein comprising a corresponding apo-**phycobiliprotein** domain, and a recombinant **phycobiliprotein** domain-bilin lyase, which components react to form the **holo-phycobiliprotein** fusion protein. Also described are **holo-phycobiliprotein** based transcription reporter cells and assays, which cells conditionally express a heterologous-to-the-cell, fluorescent, first **holo-phycobiliprotein** domain.

ACCESSION NUMBER: 2003:97917 HCAPLUS  
DOCUMENT NUMBER: 138:148684  
TITLE: Engineering of living cells for the expression of **holo-phycobiliprotein-based constructs**  
INVENTOR(S): Glazer, Alexander N.; Tooley, Aaron J.; Cai, Yuping  
PATENT ASSIGNEE(S): USA  
SOURCE: U.S. Pat. Appl. Publ., 13 pp.  
CODEN: USXXCO  
DOCUMENT TYPE: Patent

LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003027285	A1	20030206	US 2001-919486	20010731
WO 2003012448	A1	20030213	WO 2002-US24245	20020730
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			

PRIORITY APPLN. INFO.: US 2001-919486 A 20010731

L6 ANSWER 8 OF 11 HCAPLUS COPYRIGHT 2003 ACS

TI Biosynthesis of a fluorescent cyanobacterial C-phyococyanin **holo**-.alpha. subunit in a heterologous host

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phyococyanobilin, namely, heme oxygenase 1 and 3Z-phyococyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phyococyanin .alpha. subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce **holo**-CpcA with spectroscopic properties qual. and quant. similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to **holo**-CpcA. No significant bilin addn. took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF. This approach should permit incisive anal. of many remaining questions in **phycobiliprotein** biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001:705481 HCAPLUS

DOCUMENT NUMBER: 136:2664

TITLE: Biosynthesis of a fluorescent cyanobacterial C-phyococyanin **holo**-.alpha. subunit in a heterologous host

AUTHOR(S): Tooley, Aaron J.; Cai, Yuping A.; Glazer, Alexander N.

CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, Berkeley, CA, 94720-3200, USA

SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2001), 98(19), 10560-10565  
CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 9 OF 11 HCAPLUS COPYRIGHT 2003 ACS

TI Candidate genes for the phycoerythrocyanin .alpha. subunit lyase and biochemical analysis of pecE and pecF interposon mutants

AB The rod substructures of the *Anabaena* sp. PCC 7120 phycobilisome contain the light harvesting proteins C-phyococyanin and phycoerythrocyanin (PEC).

Even at low light intensities, PEC represents no more than 5% of the phycobilisome protein. The .beta. subunits of both proteins carry thioether-linked phycocyanobilin (PCB) at .beta.-Cys-82 and .beta.-Cys-155; however, C-phycocyanin has PCB at .alpha.-Cys-84 whereas PEC .alpha. subunit carries phycobiliviolin at this position. The *Anabaena* sp. PCC 7120 *pec* operon is made up of five genes. *PecB* and *pecA* encode the .beta. and .alpha. subunits of PEC, *pecC* encodes a linker polypeptide assocd. with PEC in the rod substructure, and *pecE* and *pecF* are genes of unknown function that show a high degree of homol. to *cpcE* and *cpcF*, that encode a C-phycocyanin .alpha. subunit PCB lyase (Fairchild, C. D., Zhao, J., Zhou, J., Colson, S. E., Bryant, D. A., and Glazer, A. N. (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89, 7017-7021). Insertional mutants in *pecE* and *pecF*, and an interposon mutant in which a portion of both *pecE* and *pecF* was deleted, were constructed. All three types of mutants grew 1.3 times slower than wild-type under limiting light conditions and showed a 20% redn. in the PCB content of whole cells relative to chlorophyll *a*. Holo-PEC was missing from the phycobilisomes of all three types of mutants and the level of the PEC linker polypeptide was reduced relative to the PEC linker polypeptide was reduced relative to the wild-type. However, .apprx.30% of the wild-type level of the PEC .beta. subunit was present in all of these phycobilisomes. In contrast, the PEC .alpha. subunit was barely detectable in the *pecE* and *pecF* mutants, but was present in the *pecEF* deletion mutant as a PCB-adduct in a 1:1 ratio with the PEC .beta. subunit. The identity of this "unnatural" adduct was confirmed by isolation of the subunit and amino-terminal sequencing. These biochem. results support the inference that *pecE* and *pecF* encode a PEC .alpha. subunit phycobiliviolin lyase, and, in conjunction with earlier findings, demonstrate that **phycobiliprotein** bilin lyases show high selectivity (rather than abs. specificity) for both the bilin and the polypeptide substrate.

ACCESSION NUMBER: 1995:597221 HCAPLUS  
DOCUMENT NUMBER: 123:250826  
TITLE: Candidate genes for the phycoerythrocyanin .alpha. subunit lyase and biochemical analysis of *pecE* and *pecF* interposon mutants  
AUTHOR(S): Jung, Linda J.; Chan, Crystal F.; Glazer, Alexander N.  
CORPORATE SOURCE: Department Molecular Cell Biology, University California, Berkeley, CA, 94720, USA  
SOURCE: Journal of Biological Chemistry (1995), 270(21), 12877-84  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English

L6 ANSWER 10 OF 11 SCISEARCH COPYRIGHT 2003 ISI (R)

TI Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo-alpha subunit in a heterologous host

AB The entire pathway for the synthesis of a fluorescent

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holophycobiliprotein subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid *trp-lac* (*trc*) promoter. Genes for the apoprotein (C-phycocyanin alpha subunit; *cpcA*) and the heterodimeric lyase (*cpcE* and *cpcF*) that catalyzes chromophore attachment were expressed from the *trc* promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin

addition took place in a similarly engineered E. coli strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in **phycobiliprotein** biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001:754757 SCISEARCH  
THE GENUINE ARTICLE: 472CZ  
TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo**-alpha subunit in a heterologous host  
AUTHOR: Tooley A J; Cai Y P A; Glazer A N (Reprint)  
CORPORATE SOURCE: Univ Calif Syst, Nat Res Syst, 1111 Franklin St, 6th Floor, Oakland, CA 94607 USA (Reprint); Univ Calif Berkeley, Dept Mol & Cell Biol, Berkeley, CA 94720 USA  
COUNTRY OF AUTHOR: USA  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (11 SEP 2001) Vol. 98, No. 19, pp. 10560-10565.  
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418 USA.  
ISSN: 0027-8424.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 28

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

L6 ANSWER 11 OF 11 SCISEARCH COPYRIGHT 2003 ISI (R)

TI CANDIDATE GENES FOR THE PHYCOERYTHROCYANIN ALPHA-SUBUNIT LYASE - BIOCHEMICAL-ANALYSIS OF PECE AND PECF INTERPOSON MUTANTS

AB The rod substructures of the *Anabaena* sp, PCC 7120 phycobilisome contain the light harvesting proteins C-phycocyanin and phycoerythrocyanin (PEG). Even at low light intensities, PEC represents no more than 5% of the phycobilisome protein. The beta subunits of both proteins carry thioether-linked phycocyanobilin (PCB) at beta-Cys-82 and beta-Cys-155; however, C-phycocyanin has PCB at alpha-Cys-84 whereas PEC alpha subunit carries phycobiliviolin at this position. The *Anabaena* sp, PCC 7120 pec operon is made up of five genes, pecB and pecA encode the beta and alpha subunits of PEC, pecC encodes a linker polypeptide associated with PEC in the rod substructure, and pecE and pecF are genes of unknown function that show a high degree of homology to cpcE and cpcF, that encode a C-phycocyanin alpha subunit PCB lyase (Fairchild, C, D., Zhao, J., Zhou, J., Colson, S, E., Bryant, D. A., and Glazer, A. N. (1992) Proc. Natl. Acad. Sci. U.S. A. 89, 7017-7021), Insertional mutants in pecE and pecF, and an interposon mutant in which a portion of both pecE and pecF was deleted, were constructed. All three types of mutants grew 1.3 times slower than wild-type under limiting light conditions and showed a 20% reduction in the PCB content of whole cells relative to chlorophyll alpha. **Holo**-PEC was missing from the phycobilisomes of all three types of mutants and the level of the PEC linker polypeptide was reduced relative to the wild-type. However, similar to 30% of the wild-type level of the PEC beta subunit was present in all of these phycobilisomes, In contrast, the PEC alpha subunit was barely detectable in the pecE and pecF mutants, but was present in the pecEF deletion mutant as a PCB-adduct in a 1:1 ratio with the PEC beta subunit. The identity of this 'unnatural' adduct was confirmed by isolation of the subunit and amino-terminal sequencing, These biochemical results support the inference that pecE and pecF encode a PEC a subunit phycobiliviolin lyase, and, in conjunction with earlier findings, demonstrate that **phycobiliprotein** bilin lyases show high selectivity (rather than absolute specificity) for both the bilin and the polypeptide substrate.

ACCESSION NUMBER: 95:367157 SCISEARCH

THE GENUINE ARTICLE: QZ711

TITLE: CANDIDATE GENES FOR THE PHYCOERYTHROCYANIN ALPHA-SUBUNIT LYASE - BIOCHEMICAL-ANALYSIS OF PECE AND PECF INTERPOSON MUTANTS

AUTHOR: JUNG L J; CHAN C F; GLAZER A N (Reprint)  
 CORPORATE SOURCE: UNIV CALIF BERKELEY, DEPT MOLEC & CELL BIOL, 229 STANLEY HALL, BERKELEY, CA, 94720 (Reprint); UNIV CALIF BERKELEY, DEPT MOLEC & CELL BIOL, BERKELEY, CA, 94720  
 COUNTRY OF AUTHOR: USA  
 SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (26 MAY 1995) Vol. 270, No. 21, pp. 12877-12884.  
 ISSN: 0021-9258.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: ENGLISH  
 REFERENCE COUNT: 39  
 \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

=> d his

(FILE 'HOME' ENTERED AT 12:57:47 ON 28 MAR 2003)

FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, WPIDS, BIOBUSINESS, HCAPLUS, SCISEARCH, JICST-EPLUS, FSTA' ENTERED AT 12:59:31 ON 28 MAR 2003

L1 565 S APO-ENZYME  
 L2 455 S HOLO-ENZYME  
 L3 81 S L2 AND L1  
 L4 1404 S PHYCOBILIPROTEIN  
 L5 0 S L4 AND L2  
 L6 11 S L4 AND HOLO

=> s l4 and apo  
 L7 32 L4 AND APO

=> s l6 and l7  
 L8 7 L6 AND L7

=> d l8 ti abs ibib tot

L8 ANSWER 1 OF 7 MEDLINE  
 TI Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo**-alpha subunit in a heterologous host.  
 AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phycocyanin alpha subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce **holo**-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the **apo**-CpcA was converted to **holo**-CpcA. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in **phycobiliprotein** biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001504133 MEDLINE  
 DOCUMENT NUMBER: 21438034 PubMed ID: 11553806  
 TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo**-alpha subunit in a heterologous host.  
 AUTHOR: Tooley A J; Cai Y A; Glazer A N

CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, 142 LSA no. 3200, Berkeley, CA 94720-3200, USA.  
 SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Sep 11) 98 (19) 10560-5. Journal code: 7505876. ISSN: 0027-8424.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200111  
 ENTRY DATE: Entered STN: 20010913  
 Last Updated on STN: 20011105  
 Entered Medline: 20011101

L8 ANSWER 2 OF 7 USPATFULL  
 TI Engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs  
 AB Recombinant cells which express a fluorescent **holo-phycobiliprotein** fusion protein and methods of use are described. The cells comprises a bilin, a recombinant bilin reductase, an **apo-phycobiliprotein** fusion protein precursor of the fusion protein comprising a corresponding **apo-phycobiliprotein** domain, and a recombinant **phycobiliprotein** domain-bilin lyase, which components react to form the **holo-phycobiliprotein** fusion protein. Also described are **holo-phycobiliprotein** based transcription reporter cells and assays, which cells conditionally express a heterologous-to-the-cell, fluorescent, first **holo-phycobiliprotein** domain.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2003:37640 USPATFULL  
 TITLE: Engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs  
 INVENTOR(S): Glazer, Alexander N., Berkeley, CA, UNITED STATES  
 Tooley, Aaron J., Berkeley, CA, UNITED STATES  
 Cai, Yuping, Carmel, IN, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003027285	A1	20030206
APPLICATION INFO.:	US 2001-919486	A1	20010731 (9)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	RICHARD ARON OSMAN, SCIENCE AND TECHNOLOGY LAW GROUP, 75 DENISE DRIVE, HILLSBOROUGH, CA, 94010		
NUMBER OF CLAIMS:	24		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	3 Drawing Page(s)		
LINE COUNT:	918		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 3 OF 7 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
 TI Biosynthesis of the cyanobacterial light-harvesting polypeptide phycoerythrocyanin **holo-.alpha.** subunit in a heterologous host.  
 AB The entire pathway for the biosynthesis of the phycobiliviolin-bearing His-tagged **holo-.alpha.** subunit of the cyanobacterial photosynthetic accessory protein phycoerythrocyanin was reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes required for the conversion of heme to 3Z-phycoerythrocyanin, a precursor of phycobiliviolin (namely, heme oxygenase 1 and 3Z-phycoerythrocyanin:ferredoxin oxidoreductase), were expressed from a plasmid under the control of the hybrid trp-lac (trc) promoter. Genes for the **apo**

-phycoerythrocyanin .alpha. subunit (pecA) and the heterodimeric lyase/isomerase (pecE and pecF), which catalyzes both the covalent attachment of phycocyanobilin and its concurrent isomerization to phycobiliviolin, were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used endogenous heme to produce **holo**-PecA with absorbance and fluorescence properties similar to those of the same protein produced in cyanobacteria. About two-thirds of the **apo**-PecA was converted to **holo**-PecA. No significant bilin addition took place in a similarly engineered E. coli strain that lacks pecE and pecF. By using immobilized metal affinity chromatography, both **apo**-PecA and **holo**-PecA were isolated as ternary complexes with PecE and PecF. The identities of all three components in the ternary complexes were established unambiguously by protein and tryptic peptide analyses performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

ACCESSION NUMBER: 2002295599 EMBASE  
 TITLE: Biosynthesis of the cyanobacterial light-harvesting polypeptide phycoerythrocyanin **holo**-.alpha. subunit in a heterologous host.  
 AUTHOR: Tooley A.J.; Glazer A.N.  
 CORPORATE SOURCE: A.N. Glazer, Natural Reserve System, University of California, 1111 Franklin Street, Oakland, CA 94607-5200, United States. glazer@uclink4.berkeley.edu  
 SOURCE: Journal of Bacteriology, (2002) 184/17 (4666-4671).  
 Refs: 22  
 ISSN: 0021-9193 CODEN: JOBAAY  
 COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article  
 FILE SEGMENT: 004 Microbiology  
 LANGUAGE: English  
 SUMMARY LANGUAGE: English

L8 ANSWER 4 OF 7 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
 TI Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo**-.alpha. subunit in a heterologous host.  
 AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (Synechocystis sp. PCC6803) was reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phycocyanin .alpha. subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used the cellular pool of heme to produce **holo**-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the **apo**-CpcA was converted to **holo**-CpcA. No significant bilin addition took place in a similarly engineered E. coli strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in **phycobiliprotein** biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001329835 EMBASE  
 TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo**-.alpha. subunit in a heterologous host.  
 AUTHOR: Tooley A.J.; Cai Y.A.; Glazer A.N.  
 CORPORATE SOURCE: A.N. Glazer, Natural Reserve System, University of California System, 1111 Franklin Street, Oakland, CA 94607-5200, United States. alexander.glazer@ucop.edu  
 SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (11 Sep 2001) 98/19

(10560-10565).

Refs: 30

ISSN: 0027-8424 CODEN: PNASA6

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

L8 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2003 ACS

TI Engineering of living cells for the expression of **holo-  
phycobiliprotein**-based constructs

AB Recombinant cells which express a fluorescent **holo-  
phycobiliprotein** fusion protein and methods of use are described.  
The cells comprises a bilin, a recombinant bilin reductase, an **apo-  
-phycobiliprotein** fusion protein precursor of the fusion protein  
comprising a corresponding **apo-phycobiliprotein**  
domain, and a recombinant **phycobiliprotein** domain-bilin lyase,  
which components react to form the **holo-phycobiliprotein**  
fusion protein. Also described are **holo-  
phycobiliprotein** based transcription reporter cells and assays,  
which cells conditionally express a heterologous-to-the-cell, fluorescent,  
first **holo-phycobiliprotein** domain.

ACCESSION NUMBER: 2003:97917 HCAPLUS

DOCUMENT NUMBER: 138:148684

TITLE: Engineering of living cells for the expression of  
**holo-phycobiliprotein**-based  
constructs

INVENTOR(S): Glazer, Alexander N.; Tooley, Aaron J.; Cai, Yuping

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 13 pp.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003027285	A1	20030206	US 2001-919486	20010731
WO 2003012448	A1	20030213	WO 2002-US24245	20020730
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			

PRIORITY APPLN. INFO.: US 2001-919486 A 20010731

L8 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2003 ACS

TI Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo-  
-.alpha.** subunit in a heterologous host

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phycocyanin .alpha. subunit; cpcA) and the heterodimeric

lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used the cellular pool of heme to produce **holo**-CpcA with spectroscopic properties qual. and quant. similar to those of the same protein produced endogenously in cyanobacteria. About a third of the **apo**-CpcA was converted to **holo**-CpcA. No significant bilin addn. took place in a similarly engineered E. coli strain that lacks cpcE and cpcF. This approach should permit incisive anal. of many remaining questions in **phycobiliprotein** biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001:705481 HCAPLUS  
DOCUMENT NUMBER: 136:2664  
TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo**-alpha subunit in a heterologous host  
AUTHOR(S): Tooley, Aaron J.; Cai, Yuping A.; Glazer, Alexander N.  
CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, Berkeley, CA, 94720-3200, USA  
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2001), 98(19), 10560-10565  
CODEN: PNASA6; ISSN: 0027-8424  
PUBLISHER: National Academy of Sciences  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 7 OF 7 SCISEARCH COPYRIGHT 2003 ISI (R)

TI Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo**-alpha subunit in a heterologous host

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (Synechocystis sp. PCC6803) was reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phycocyanin alpha subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used the cellular pool of heme to produce **holo**-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the **apo**-CpcA was converted to **holo**-CpcA. No significant bilin addition took place in a similarly engineered E. coli strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in **phycobiliprotein** biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

---

ACCESSION NUMBER: 2001:754757 SCISEARCH  
THE GENUINE ARTICLE: 472CZ  
TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin **holo**-alpha subunit in a heterologous host  
AUTHOR: Tooley A J; Cai Y P A; Glazer A N (Reprint)  
CORPORATE SOURCE: Univ Calif Syst, Nat Res Syst, 1111 Franklin St, 6th Floor, Oakland, CA 94607 USA (Reprint); Univ Calif Berkeley, Dept Mol & Cell Biol, Berkeley, CA 94720 USA  
COUNTRY OF AUTHOR: USA  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (11 SEP 2001) Vol. 98, No. 19, pp. 10560-10565.  
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW,

WASHINGTON, DC 20418 USA.

ISSN: 0027-8424.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 28

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

=> d his

(FILE 'HOME' ENTERED AT 12:57:47 ON 28 MAR 2003)

FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, WPIDS, BIOBUSINESS, HCAPLUS,  
SCISEARCH, JICST-EPLUS, FSTA' ENTERED AT 12:59:31 ON 28 MAR 2003

L1	565 S APO-ENZYME
L2	455 S HOLO-ENZYME
L3	81 S L2 AND L1
L4	1404 S PHYCOBILIPROTEIN
L5	0 S L4 AND L2
L6	11 S L4 AND HOLO
L7	32 S L4 AND APO
L8	7 S L6 AND L7

CC 7-2 (Enzymes)  
 AB Unavailable  
 ST phytochromobilin synthase oat; Avena phytochromobilin synthase  
 IT Oat  
     (purifn. and characterization of phytochromobilin synthase from Avena sativa)  
 IT **138263-99-7P**, Phytochromobilin synthase  
 RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)  
     (purifn. and characterization of phytochromobilin synthase from Avena sativa)

L92 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2003 ACS  
 AN 1992:36617 HCAPLUS  
 DN 116:36617  
 TI **Holophytochrome** assembly. Coupled assay for phytochromobilin synthase in organello  
 AU Terry, Matthew J.; Lagarias, J. Clark  
 CS Dep. Biochem. Biophys., Univ. California, Davis, CA, 95616, USA  
 SO Journal of Biological Chemistry (**1991**), 266(33), 22215-21  
 CODEN: JBCHA3; ISSN: 0021-9258  
 DT Journal  
 LA English  
 CC 7-1 (Enzymes)  
     Section cross-reference(s): 6, 11

AB Utilizing an in vitro coupled assay system, the authors show that isolated plastids from cucumber cotyledons convert the linear tetrapyrrole biliverdin IX.alpha. to the free phytochrome chromophore, phytochromobilin, which assembles with oat apophytochrome to yield photoactive **holoprotein**. The spectral properties of this synthetic phytochrome are indistinguishable from those of the natural photoreceptor. The plastid-dependent biliverdin conversion activity is strongly stimulated by both NADPH and ATP. Substitution of the nonnatural XIII.alpha. isomer of biliverdin for the IX.alpha. isomer affords a synthetic **holophytochrome** adduct with blue-shifted difference spectra. These results, together with expts. using boiled plastids, indicate that phytochromobilin synthesis from biliverdin is enzyme-mediated. Expts. where NADPH (and ATP) levels in intact developing chloroplasts are manipulated by feeding the metabolites 3-phosphoglycerate, dihydroxyacetone phosphate, and glucose 6-phosphate or by illumination with white light, support the hypothesis that the enzyme that accomplishes this conversion, phytochromobilin synthase, is plastid-localized. It is therefore likely that all of the enzymes of the phytochrome chromophore biosynthetic pathway reside in the plastid.

ST phytochrome assembly etioplast chloroplast plant; phytochromobilin synthase detection plastid plant  
 IT Oat  
     (apophytochrome of seedling of, phytochromobilin of cucumber cotyledon and synthetic biliverdin XIII.alpha.-contg. phytochromobilin-binding by)

---

IT **Phytochromes**  
 RL: SPN (Synthetic preparation); PREP (Preparation)  
     (biliverdin XIII.alpha.-contg., prepn. and properties of)

IT Light  
     (phytochrome formation by cucumber cotyledon chloroplast response to)

IT Chloroplast  
     (phytochrome synthase detection in and phytochrome assembly by, of plant)

IT Cucumber  
     (phytochromobilin of plastid of, formation of and oat apophytochrome binding by)

IT Plant

(phytochromobilin synthase in plastids of and phytochrome assembly by)

IT **Phytochromes**  
RL: FORM (Formation, nonpreparative)  
(Pr, formation of, by plant plastid)

IT **Phytochromes**  
RL: ANST (Analytical study)  
(apo-, phytochromobilin of cucumber cotyledon and synthetic biliverdin  
XIII.alpha.-contg. phytochromobilin coupling with, of oat seedling)

IT Plastid  
(etio-, phytochrome synthase detection in and phytochrome assembly by,  
of plant)

IT **138263-99-7**, Phytochromobilin synthase  
RL: ANT (Analyte); ANST (Analytical study)  
(detection of, in chloroplast and etioplast of plant, by coupled assay)

IT 78249-71-5, Phytochromobilin  
RL: ANST (Analytical study)  
(formation of and apophytochrome of plant coupling with, in plant  
chloroplast and etioplast)

IT 56-65-5, 5'-ATP, biological studies  
RL: BIOL (Biological study)  
(phytochromobilin synthase of plant chloroplast and etioplast  
stimulation by)

IT **114-25-0**, Biliverdin IX.alpha. 28022-06-2, Biliverdin  
XIII.alpha.  
RL: RCT (Reactant); RACT (Reactant or reagent)  
(reaction of, with phytochromobilin synthase of plant etioplast and  
chloroplast)

=> sel hit rn  
E9 THROUGH E15 ASSIGNED

=> fil reg  
FILE 'REGISTRY' ENTERED AT 13:56:10 ON 27 MAR 2003  
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.  
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.  
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Property values tagged with IC are from the ZIC/VINITI data file  
provided by InfoChem.

STRUCTURE FILE UPDATES: 26 MAR 2003 HIGHEST RN 500755-46-4  
DICTIONARY FILE UPDATES: 26 MAR 2003 HIGHEST RN 500755-46-4

TSCA INFORMATION NOW CURRENT THROUGH MAY 20, 2002

Please note that search-term pricing does apply when  
conducting SmartSELECT searches.

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~~Crossover limits have been increased. See HELP CROSSOVER for details.~~

Experimental and calculated property data are now available. See HELP  
PROPERTIES for more information. See STNote 27, Searching Properties  
in the CAS Registry File, for complete details:  
<http://www.cas.org/ONLINE/STN/STNOTES/stnotes27.pdf>

=> s e9-e15 not 180,181  
1 138263-99-7/BI  
(138263-99-7/RN)  
1 114-25-0/BI  
(114-25-0/RN)  
1 18097-67-1/BI  
(18097-67-1/RN)

1 20298-86-6/BI  
(20298-86-6/RN)  
1 347401-12-1/BI  
(347401-12-1/RN)  
1 347401-20-1/BI  
(347401-20-1/RN)  
1 347401-21-2/BI  
(347401-21-2/RN)  
L93 3 (138263-99-7/BI OR 114-25-0/BI OR 18097-67-1/BI OR 20298-86-6/BI  
OR 347401-12-1/BI OR 347401-20-1/BI OR 347401-21-2/BI) NOT  
(L80 OR L81)

=> d ide can tot

L93 ANSWER 1 OF 3 REGISTRY COPYRIGHT 2003 ACS  
RN **347401-20-1** REGISTRY  
CN Oxidoreductase, ferredoxin:15,16-dihydrobiliverdin (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN Ferredoxin:15,16-dihydrobiliverdin oxidoreductase  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
3 REFERENCES IN FILE CA (1962 TO DATE)  
3 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 137:365572

REFERENCE 2: 136:50278

REFERENCE 3: 135:73274

L93 ANSWER 2 OF 3 REGISTRY COPYRIGHT 2003 ACS  
RN **138263-99-7** REGISTRY  
CN Synthase, phytochromobilin (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN Ferredoxin:3Z-phytochromobilin oxidoreductase  
CN Phytochromobilin synthase  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: AGRICOLA, BIOSIS, CA, CAPLUS

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
8 REFERENCES IN FILE CA (1962 TO DATE)  
8 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 137:365572

REFERENCE 2: 136:50278

REFERENCE 3: 135:207297

REFERENCE 4: 135:118588

REFERENCE 5: 135:73274

REFERENCE 6: 132:204723

REFERENCE 7: 125:190274

REFERENCE 8: 116:36617

L93 ANSWER 3 OF 3 REGISTRY COPYRIGHT 2003 ACS

RN 114-25-0 REGISTRY

CN 21H-Biline-8,12-dipropionic acid, 3,18-diethenyl-1,19,22,24-tetrahydro-2,7,13,17-tetramethyl-1,19-dioxo- (9CI) (CA INDEX NAME)

OTHER CA INDEX NAMES:

CN Biline-8,12-dipropionic acid, 1,19,22,24-tetrahydro-2,7,13,17-tetramethyl-1,19-dioxo-3,18-divinyl- (8CI)

CN Pyrrole-3-propionic acid, 2-[[3-(2-carboxyethyl)-4-methyl-5-[(3-methyl-5-oxo-4-vinyl-3-pyrrolin-2-ylidene)methyl]-2H-pyrrol-2-ylidene)methyl]-4-methyl-5-[(4-methyl-5-oxo-3-vinyl-3-pyrrolin-2-ylidene)methyl]- (7CI)

OTHER NAMES:

CN Biliverdin

CN Biliverdin IX.alpha.

CN Biliverdine

CN Dehydrobilirubin

CN Oocyan

CN Protobiliverdin IX.alpha.

CN Uteroverdine

FS STEREOSEARCH

DR 493-89-0, 27818-05-9, 29575-14-2, 189246-93-3

MF C33 H34 N4 O6

GI COM

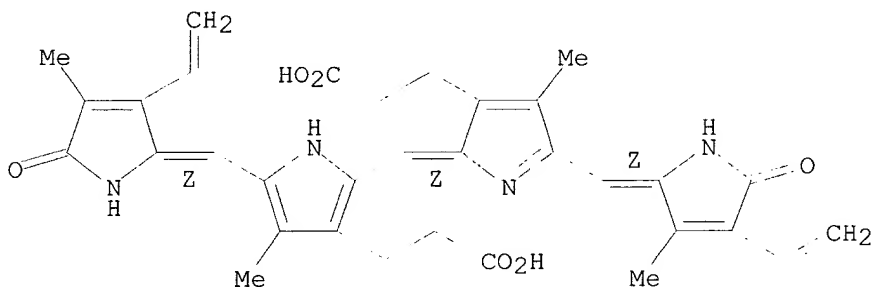
LC STN Files: AGRICOLA, ANABSTR, BEILSTEIN\*, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA, CANCERLIT, CAOLD, CAPLUS, CASREACT, CHEMLIST, CSCHEM, DDFU, DRUGU, EMBASE, GMELIN\*, HODOC\*, IPA, MEDLINE, MRCK\*, TOXCENTER, USPATFULL

(\*File contains numerically searchable property data)

Other Sources: EINECS\*\*, NDSL\*\*, TSCA\*\*

(\*\*Enter CHEMLIST File for up-to-date regulatory information)

Double bond geometry as shown.



\*\*PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT\*\*

~~614 REFERENCES IN FILE CA (1962 TO DATE)~~

43 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA

614 REFERENCES IN FILE CAPLUS (1962 TO DATE)

1 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

REFERENCE 1: 138:182973

REFERENCE 2: 138:165503

REFERENCE 3: 138:148684

REFERENCE 4: 138:120036

REFERENCE 5: 138:39129

REFERENCE 6: 138:34738  
 REFERENCE 7: 138:21090  
 REFERENCE 8: 138:2706  
 REFERENCE 9: 138:174  
 REFERENCE 10: 137:365572

=> d his

(FILE 'HOME' ENTERED AT 12:54:49 ON 27 MAR 2003)  
 SET COST OFF

FILE 'HCAPLUS' ENTERED AT 12:55:07 ON 27 MAR 2003

E US20030027285/PN  
 L1 1 S E3  
 E GLAZER A/AU  
 L2 262 S E3,E7,E11,E13,E14  
 E TOOLEY A/AU  
 L3 3 S E4  
 E CAI Y/AU  
 L4 253 S E3-E18  
 E CAI YU/AU  
 L5 30 S E3,E10  
 E CAI YUPING/AU  
 L6 15 S E3  
 L7 4 S E4  
 E BILIPROTEIN/CT  
 E E4+ALL  
 L8 6658 S E7,E5+NT  
 L9 2 S HOLOPHYCOBILIPROTEIN OR HOLO() (PHYCOBILIPROTEIN OR PHYCOBILI#

FILE 'REGISTRY' ENTERED AT 13:01:53 ON 27 MAR 2003

L10 1 S 9059-22-7  
 L11 1 S 14875-96-8

FILE 'HCAPLUS' ENTERED AT 13:02:57 ON 27 MAR 2003

L12 9974 S L10 OR L11  
 L13 2648 S HEME OXYGENASE  
 L14 30884 S HEME OR PROTOHEME OR REDUCED HEMATIN OR HEM FE OR FERROHEME O  
 L15 66 S L8 AND L12-L14  
 L16 2 S L9 AND L15  
 L17 813 S PHYCOBILIPROTEIN  
 L18 6658 S L8,L9  
 L19 66 S L18 AND L12-L14  
 L20 ~~6 S L19 AND (RECOMBIN? OR CONTRUCT)~~  
 L21 126 S L2-L7 AND L8,L9,L17,L18  
 L22 4 S L12-L14 AND L21  
 L23 302 S BILIN  
 L24 336 S ?PHYCOCYANOBILIN?  
 L25 816 S ?PHYCOBILIPROTEIN?  
 L26 122 S ?PHYCOERYTHROCYANIN?  
 L27 12 S ?PHYCOBILIVIOLIN?  
 L28 173 S ?PHYCOERYTHROBILIN?  
 L29 2074 S ?PHYCOERYTHRIN?  
 L30 112 S L2-L7 AND L23-L29  
 L31 4 S L12-L14 AND L30  
 L32 7 S L1,L9,L16,L20,L22,L31  
 L33 6 S L32 NOT PHARMACEUTICAL/TI

SEL RN L1

FILE 'REGISTRY' ENTERED AT 13:20:58 ON 27 MAR 2003

L34 9 S E1-E9  
L35 1 S 20298-86-6  
L36 1 S 93527-36-7  
L37 45 S C33H38N4O6/MF AND NC4/ES AND 4/NR  
L38 44 S L37 AND BILIN?  
L39 7 S L38 AND 8 12 DIPROPANOIC AND 18 ETHYL 3 ETHYLIDENE  
L40 7 S L39 AND 2 7 13 17 TETRAMETHYL 1 19 DIOXO  
L41 7 S L35,L36,L40  
L42 1 S 347401-12-1  
E PHYCOCYANOBILIN/CN  
L43 1 S E8  
E APO-PHYCOBILIPROTEIN/CN  
E PHYCOCYANIN/CN  
L44 116 S PHYCOCYANIN (L) ALPHA (L) SUBUNIT  
L45 1 S 168680-20-4  
L46 1 S 124861-40-1  
L47 1 S 18097-67-1  
L48 174 S C33H38N4O6/MF  
L49 45 S L48 AND NC4/ES AND 4/NR  
L50 3 S L49 AND BILINE 8 12 DIPROPANOIC AND 18 ETHENYL 3 ETHYLIDENE A  
L51 3 S L47,L50  
L52 1 S 347401-21-2

FILE 'HCAPLUS' ENTERED AT 13:37:26 ON 27 MAR 2003

L53 350 S L41 OR PHYCOCYANOBILIN  
L54 8 S L42 OR PHYCOCYANOBILIN(S) FERREDOXIN(S) OXIDOREDUCTASE  
L55 8 S L45 OR PHYCOERYTHROCYANIN LYASE  
L56 12 S L46 OR PHYCOBILIVIOLIN  
L57 25 S L12-L14 AND L53  
L58 43 S L54-L57  
L59 31 S L58 AND L8  
L60 2 S L59 AND L9

FILE 'REGISTRY' ENTERED AT 13:41:42 ON 27 MAR 2003

L61 1 S 144378-42-7

FILE 'HCAPLUS' ENTERED AT 13:42:39 ON 27 MAR 2003

L62 6 S L61 OR PHYCOCYANIN (S) ALPHA(S) SUBUNIT (S) PHYCOCYANOBILIN LY  
L63 28 S L54-L56,L62  
L64 7 S L63 AND L12-L14  
L65 76 S HOLO? AND L8,L53-L60,L62-L64  
L66 71 S L65 AND (PD<=20010731 OR PRD<=20010731 OR AD<=20010731)  
L67 7 S L54,L55,L62 AND L66  
L68 19 S L54,L55,L62  
L69 19 S L67,L68  
L70 36 S L2-L7 AND L53-L60,L62-L63  
L71 4 S L70 AND L66  
L72 5 S L70 AND L65  
L73 5 S L71,L72  
E GENETIC ENGINEERING/CT  
E E3+ALL  
L74 79989 S E2+NT  
L75 10 S L74 AND L65  
L76 12 S L73,L75  
L77 263 S L74 AND L12-L14  
L78 8 S L77 AND L23-L29,L53-L56,L62,L63  
L79 14 S L76,L78

FILE 'HCAPLUS' ENTERED AT 13:50:25 ON 27 MAR 2003

SEL HIT RN

FILE 'REGISTRY' ENTERED AT 13:50:40 ON 27 MAR 2003  
L80 8 S E1-E8  
L81 10 S L41,L42,L43,L45-L47,L51,L52,L61 NOT L80

FILE 'HCAPLUS' ENTERED AT 13:52:50 ON 27 MAR 2003  
L82 7 S L42,L52  
L83 1 S L82 NOT L79  
L84 1 S L83 AND L1-L33,L53-L60,L62-L79,L82-L83

FILE 'REGISTRY' ENTERED AT 13:54:24 ON 27 MAR 2003  
L85 2 S 138263-99-7 OR 347401-20-1  
L86 2 S 114-25-0 OR 18097-67-1

FILE 'HCAPLUS' ENTERED AT 13:54:36 ON 27 MAR 2003  
L87 8 S L85  
L88 753 S L86  
L89 5 S L87 AND L1-L33,L53-L60,L62-L69,L82-L84,L87,L88 NOT L79  
L90 5 S L84,L89  
L91 2 S L90 AND L88  
L92 5 S L90,L91

FILE 'HCAPLUS' ENTERED AT 13:55:52 ON 27 MAR 2003  
SEL HIT RN

FILE 'REGISTRY' ENTERED AT 13:56:10 ON 27 MAR 2003  
L93 3 S E9-E15 NOT L80,L81

=> fil biosis

FILE 'BIOSIS' ENTERED AT 14:00:43 ON 27 MAR 2003  
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FILE COVERS 1969 TO DATE.  
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT  
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 26 March 2003 (20030326/ED)

=> d all tot 1105

L105 ANSWER 1 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
AN 2002:464629 BIOSIS  
DN PREV200200464629  
TI Biosynthesis of the cyanobacterial light-harvesting polypeptide  
**phycoerythrocyanin** holo-alpha subunit in a heterologous  
host.  
AU **Tooley, Aaron J.; Glazer, Alexander N. (1)**  
CS (1) Natural Reserve System, University of California, 1111 Franklin  
Street, 6th Floor, Oakland, CA, 94607-5200: glazer@uclink4.berkeley.edu  
USA  
SO Journal of Bacteriology, (September, 2002) Vol. 184, No. 17, pp.  
4666-4671. <http://intl-jb.asm.org/>. print.  
ISSN: 0021-9193.  
DT Article  
LA English  
AB The entire pathway for the biosynthesis of the **phycobiliviolin**  
-bearing His-tagged holo-alpha subunit of the cyanobacterial  
photosynthetic accessory protein **phycoerythrocyanin** was  
reconstituted in Escherichia coli. Cyanobacterial genes encoding enzymes  
required for the conversion of heme to 3Z-**phycocyanobilin**, a  
precursor of **phycobiliviolin** (namely, heme oxygenase 1 and 3Z-  
**phycocyanobilin**:ferredoxin oxidoreductase), were expressed from a

plasmid under the control of the hybrid trp-lac (trc) promoter. Genes for the apo-**phycoerythrocyanin** alpha subunit (pecA) and the heterodimeric lyase/isomerase (pecE and pecF), which catalyzes both the covalent attachment of **phycocyanobilin** and its concurrent isomerization to **phycobiliviolin**, were expressed from the trc promoter on a second plasmid. Upon induction, recombinant E. coli used endogenous heme to produce **holo**-PecA with absorbance and fluorescence properties similar to those of the same protein produced in cyanobacteria. About two-thirds of the apo-PecA was converted to **holo**-PecA. No significant **bilin** addition took place in a similarly engineered E. coli strain that lacks pecE and pecF. By using immobilized metal affinity chromatography, both apo-PecA and **holo**-PecA were isolated as ternary complexes with PecE and PecF. The identities of all three components in the ternary complexes were established unambiguously by protein and tryptic peptide analyses performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

- CC Genetics and Cytogenetics - General \*03502  
Physiology and Biochemistry of Bacteria \*31000  
Genetics of Bacteria and Viruses \*31500
- BC Enterobacteriaceae 06702  
Nostocaceae 09241
- IT Major Concepts  
Molecular Genetics (Biochemistry and Molecular Biophysics)
- IT Chemicals & Biochemicals  
apo-PecA protein; **holo**-PecA protein;  
**phycoerythrocyanin**: biosynthesis, **holo**-alpha subunit,  
light-harvesting polypeptide
- IT Methods & Equipment  
immobilized metal affinity chromatography: purification method;  
matrix-assisted laser desorption ionization-time of flight mass  
spectrometry: analytical method; tryptic peptide analysis: analytical  
method
- ORGN Super Taxa  
Enterobacteriaceae: Facultatively Anaerobic Gram-Negative Rods,  
Eubacteria, Bacteria, Microorganisms; Nostocaceae: Nostocales,  
Cyanobacteria, Oxygenic Photosynthetic Bacteria, Eubacteria, Bacteria,  
Microorganisms
- ORGN Organism Name  
Anabaena sp. (Nostocaceae): strain-PCC7120; Escherichia coli  
(Enterobacteriaceae): DH5-alpha
- ORGN Organism Superterms  
Bacteria; Cyanobacteria; Eubacteria; Microorganisms
- GEN Anabaena pecA gene [Anabaena apo-**phycoerythrocyanin**  
alpha-subunit gene] (Nostocaceae); Anabaena pecE gene (Nostocaceae);  
Anabaena pecF gene (Nostocaceae)

L105 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AN 2001:482056 BIOSIS

DN PREV200100482056

TI Biosynthesis of a fluorescent cyanobacterial C-**phycocyanin**  
**holo**-alpha subunit in a heterologous host.

AU **Tooley, Aaron J.; Cai, Yuping A.; Glazer, Alexander N. (1)**

CS (1) Natural Reserve System, University of California System, 1111 Franklin  
Street, 6th Floor, Oakland, CA, 94607-5200: alexander.glazer@ucop.edu USA

SO Proceedings of the National Academy of Sciences of the United States of  
America, (September 11, 2001) Vol. 98, No. 19, pp. 10560-10565. print.  
ISSN: 0027-8424.

DT Article

LA English

SL English

AB The entire pathway for the synthesis of a fluorescent

*Good date*

**holophycobiliprotein** subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-**phycocyanobilin**, namely, heme oxygenase 1 and 3Z-**phycocyanobilin**:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-**phycocyanin** alpha subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce **holo**-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to **holo**-CpcA. No significant **bilin** addition took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in **phycobiliprotein** biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

- CC Genetics and Cytogenetics - General \*03502  
 Biochemical Studies - General \*10060  
 Physiology and Biochemistry of Bacteria \*31000  
 Genetics of Bacteria and Viruses \*31500
- BC Enterobacteriaceae 06702  
 Chroococcales 09210
- IT Major Concepts  
 Biochemistry and Molecular Biophysics; Molecular Genetics (Biochemistry and Molecular Biophysics)
- IT Chemicals & Biochemicals  
 3Z-**phycocyanobilin**: chromophore; 3Z-**phycocyanobilin**:ferredoxin oxidoreductase; C-**phycocyanin**: biosynthesis, fluorescent, **holo**-alpha subunit; heme oxygenase 1;  
**holophycobiliprotein**
- IT Miscellaneous Descriptors  
 photosynthesis
- ORGN Super Taxa  
 Chroococcales: Cyanobacteria, Oxygenic Photosynthetic Bacteria, Eubacteria, Bacteria, Microorganisms; Enterobacteriaceae: Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
- ORGN Organism Name  
*Escherichia coli* (Enterobacteriaceae): expression system; *Synechocystis* sp. PCC6803 (Chroococcales)
- ORGN Organism Superterms  
 Bacteria; Cyanobacteria; Eubacteria; Microorganisms
- RN 93527-36-7 (3Z-**PHYCOCYANOBILIN**)
- GEN *Synechocystis* cpcA gene [*Synechocystis* C-**phycocyanin** alpha subunit gene] (Chroococcales); *Synechocystis* cpcE gene (Chroococcales); *Synechocystis* cpcF gene (Chroococcales); trp-lac hybrid gene: promoter

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L105 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

- AN 1995:320261 BIOSIS
- DN PREV199598334561
- TI Candidate genes for the **phycoerythrocyanin** alpha subunit lyase: Biochemical analysis of pecE and pecF interposon mutants.
- AU Jung, Linda J.; Chan, Crystal F.; Glazer, Alexander N. (1)
- CS (1) MCB: Stanley/Donner ASU, 229 Stanley Hall 3206, Univ. Calif., Berkeley, CA 94720-3206 USA
- SO Journal of Biological Chemistry, (1995) Vol. 270, No. 21, pp. 12877-12884. ISSN: 0021-9258.
- DT Article
- LA English
- AB The rod substructures of the *Anabaena* sp. PCC 7120 **phycobilisome** contain the light harvesting proteins C-**phycocyanin** and

**phycoerythrocyanin** (PEC). Even at low light intensities, PEC represents no more than 5% of the **phycobilisome** protein. The beta subunits of both proteins carry thioether-linked **phycocyanobilin** (PCB) at beta-Cys-82 and beta-Cys-155; however, C-**phycocyanin** has PCB at alpha-Cys-84 whereas PEC a subunit carries **phycobiliviolin** at this position. The *Anabaena* sp. PCC 7120 *pec* operon is made up of five genes. *PecB* and *pecA* encode the beta and alpha subunits of PEC, *pecC* encodes a linker polypeptide associated with PEC in the rod substructure, and *pecE* and *pecF* are genes of unknown function that show a high degree of homology to *cpcE* and *cpcF*, that encode a C-**phycocyanin** a subunit PCB lyase (Fairchild, C. D., Zhao, J., Zhou, J., Colson, S. E., Bryant, D. A., and Glazer, A. N. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 7017-7021). Insertional mutants in *pecE* and *pecF*, and an interposon mutant in which a portion of both *pecE* and *pecF* was deleted, were constructed. All three types of mutants grew 1.3 times slower than wild-type under limiting light conditions and showed a 20% reduction in the PCB content of whole cells relative to chlorophyll alpha. **Holo-PEC** was missing from the **phycobilisomes** of all three types of mutants and the level of the PEC linker polypeptide was reduced relative to the wild-type. However, approx 30% of the wild-type level of the PEC beta subunit was present in all of these **phycobilisomes**. In contrast, the PEC a subunit was barely detectable in the *pecE* and *pecF* mutants, but was present in the *pecEF* deletion mutant as a PCB-adduct in a 1:1 ratio with the PEC beta subunit. The identity of this "unnatural" adduct was confirmed by isolation of the subunit and amino-terminal sequencing. These biochemical results support the inference that *pecE* and *pecF* encode a PEC alpha subunit **phycobiliviolin** lyase, and, in conjunction with earlier findings, demonstrate that **phycobiliprotein bilin** lyases show high selectivity (rather than absolute specificity) for both the **bilin** and the polypeptide substrate.

CC Biochemical Studies - Proteins, Peptides and Amino Acids \*10064  
 Biochemical Studies - Porphyrins and Bile Pigments \*10065  
 Biophysics - Molecular Properties and Macromolecules \*10506  
 Enzymes - Chemical and Physical \*10806  
 Physiology and Biochemistry of Bacteria \*31000  
 Genetics of Bacteria and Viruses \*31500  
 BC Nostocaceae \*09241  
 IT Major Concepts  
     Biochemistry and Molecular Biophysics; Enzymology (Biochemistry and  
     Molecular Biophysics); Genetics; Physiology  
 IT Chemicals & Biochemicals  
     LYASE; LYASES  
 IT Miscellaneous Descriptors  
     **PHYCOBILIPROTEIN BILIN LYASES;**  
     **PHYCOBILISOMES**  
 ORGN Super Taxa  
     Nostocaceae: Cyanobacteria, Eubacteria, Bacteria  
 ORGN Organism Name  
     Anabaena (Nostocaceae)  


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 ORGN Organism Superterms  
     bacteria; cyanobacteria; eubacteria; microorganisms  
 RN 9055-04-3 (LYASE)  
     9055-04-3D (LYASES)  
 L105 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
 AN 1992:506225 BIOSIS  
 DN BA94:124750  
 TI **PHYCOCYANIN ALPHA-SUBUNIT PHYCOCYANOBILIN LYASE.**  
 AU FAIRCHILD C D; ZHAO J; ZHOU J; COLSON S E; BRYANT D A; GLAZER A N  
 CS MCB: STANLEY/DONNER ASU, 229 STANLEY HALL, UNIV. CALIF., BERKELEY, CALIF.  
     94720.  
 SO PROC NATL ACAD SCI U S A, (1992) 89 (15), 7017-7021.

CODEN: PNASA6. ISSN: 0027-8424.

FS BA; OLD

LA English

AB **Phycobiliproteins**, unlike other light-harvesting proteins involved in photosynthesis, bear covalently attached chromophores. The **bilin** chromophores are attached through thioether bonds to cysteine residues. The cyanobacterium *Synechococcus* sp. PCC 7002 has eight distinct **bilin** attachment sites on seven polypeptides, all of which carry the same chromophore, **phycocyanobilin**. When two genes in the **phycocyanin** operon of this organisms, *cpcE* and *cpcF*, are inactivated by insertion, together or separately, the surprising result is elimination of correct **bilin** attachment at only one site, that on the .alpha. subunit of **phycocyanin**. We have overproduced *CpcE* and *CpcF* in *Escherichia coli*. In vitro, these proteins catalyze the attachment of **phycocyanobilin** to the .alpha. subunit of **apophycocyanin** at the appropriate site, .alpha. Cys-84, to form the correct adduct. *CpcE* and *CpcF* also efficiently catalyze the reverse reaction, in which the **bilin** from **holo**-.alpha. subunit is transferred either to the apo-.alpha. subunit of the same C-**phycocyanin** or to the apo-.alpha. subunit of a heterologous C-**phycocyanin**. The forward and reverse reactions each require both *CpcE* and *CpcF* and are specific for the .alpha.-Cys-84 position. **Phycocyanobilin** is the immediate precursor of the protein-bound **bilin**.

CC Biochemical Studies - Nucleic Acids, Purines and Pyrimidines 10062  
 Biochemical Studies - Proteins, Peptides and Amino Acids 10064  
 Biochemical Studies - Porphyrins and Bile Pigments 10065  
 Biophysics - Molecular Properties and Macromolecules 10506  
 Enzymes - Chemical and Physical \*10806  
 Enzymes - Physiological Studies \*10808  
 Metabolism - Proteins, Peptides and Amino Acids \*13012  
 Metabolism - Porphyrins and Bile Pigments \*13013  
 Physiology and Biochemistry of Bacteria \*31000  
 Genetics of Bacteria and Viruses \*31500  
 Plant Physiology, Biochemistry and Biophysics - Photosynthesis 51506  
 BC Enterobacteriaceae 06702  
 Chroococcales 09210  
 IT Miscellaneous Descriptors  
 ESCHERICHIA-COLI SYNECHOCOCCUS CPCE GENE CPCF GENE  
 APOPHYCOCYANIN ALPHA SUBUNIT ATTACHMENT SITE  
 RN 144378-42-7 (PHYCOCYANIN ALPHA-SUBUNIT PHYCOCYANOBILIN  
 LYASE)

=&gt; d his

(FILE 'HOME' ENTERED AT 12:54:49 ON 27 MAR 2003)  
 SET COST OFF

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FILE 'HCAPLUS' ENTERED AT 12:55:07 ON 27 MAR 2003

E US20030027285/PN  
 L1 1 S E3  
 E GLAZER A/AU  
 L2 262 S E3,E7,E11,E13,E14  
 E TOOLEY A/AU  
 L3 3 S E4  
 E CAI Y/AU  
 L4 253 S E3-E18  
 E CAI YU/AU  
 L5 30 S E3,E10  
 E CAI YUPING/AU  
 L6 15 S E3  
 L7 4 S E4

E BILIPROTEIN/CT  
 E E4+ALL  
 L8 6658 S E7,E5+NT  
 L9 2 S HOLOPHYCOBILIPROTEIN OR HOLO() (PHYCOBILIPROTEIN OR PHYCOBILI#

FILE 'REGISTRY' ENTERED AT 13:01:53 ON 27 MAR 2003

L10 1 S 9059-22-7  
 L11 1 S 14875-96-8

FILE 'HCAPLUS' ENTERED AT 13:02:57 ON 27 MAR 2003

L12 9974 S L10 OR L11  
 L13 2648 S HEME OXYGENASE  
 L14 30884 S HEME OR PROTOHEME OR REDUCED HEMATIN OR HEM FE OR FERROHEME O  
 L15 66 S L8 AND L12-L14  
 L16 2 S L9 AND L15  
 L17 813 S PHYCOBILIPROTEIN  
 L18 6658 S L8,L9  
 L19 66 S L18 AND L12-L14  
 L20 6 S L19 AND (RECOMBIN? OR CONTRUCT)  
 L21 126 S L2-L7 AND L8,L9,L17,L18  
 L22 4 S L12-L14 AND L21  
 L23 302 S BILIN  
 L24 336 S ?PHYCOCYANOBILIN?  
 L25 816 S ?PHYCOBILIPROTEIN?  
 L26 122 S ?PHYCOERYTHROCYANIN?  
 L27 12 S ?PHYCOBILIVIOLIN?  
 L28 173 S ?PHYCOERYTHROBILIN?  
 L29 2074 S ?PHYCOERYTHRIN?  
 L30 112 S L2-L7 AND L23-L29  
 L31 4 S L12-L14 AND L30  
 L32 7 S L1,L9,L16,L20,L22,L31  
 L33 6 S L32 NOT PHARMACEUTICAL/TI  
 SEL RN L1

FILE 'REGISTRY' ENTERED AT 13:20:58 ON 27 MAR 2003

L34 9 S E1-E9  
 L35 1 S 20298-86-6  
 L36 1 S 93527-36-7  
 L37 45 S C33H38N4O6/MF AND NC4/ES AND 4/NR  
 L38 44 S L37 AND BILIN?  
 L39 7 S L38 AND 8 12 DIPROPANOIC AND 18 ETHYL 3 ETHYLIDENE  
 L40 7 S L39 AND 2 7 13 17 TETRAMETHYL 1 19 DIOXO  
 L41 7 S L35,L36,L40  
 L42 1 S 347401-12-1  
 E PHYCOCYANOBILIN/CN  
 L43 1 S E8  
 E APO-PHYCOBILIPROTEIN/CN  
 E PHYCOCYANIN/CN  
 L44 116 S PHYCOCYANIN (L) ALPHA (L) SUBUNIT  
 L45 1 S 168680-20-4  
 L46 1 S 124861-40-1  
 L47 1 S 18097-67-1  
 L48 174 S C33H38N4O6/MF  
 L49 45 S L48 AND NC4/ES AND 4/NR  
 L50 3 S L49 AND BILINE 8 12 DIPROPANOIC AND 18 ETHENYL 3 ETHYLIDENE A  
 L51 3 S L47,L50  
 L52 1 S 347401-21-2

FILE 'HCAPLUS' ENTERED AT 13:37:26 ON 27 MAR 2003

L53 350 S L41 OR PHYCOCYANOBILIN  
 L54 8 S L42 OR PHYCOCYANOBILIN(S) FERREDOXIN(S) OXIDOREDUCTASE  
 L55 8 S L45 OR PHYCOERYTHROCYANIN LYASE  
 L56 12 S L46 OR PHYCOBILIVIOLIN

L57 25 S L12-L14 AND L53  
L58 43 S L54-L57  
L59 31 S L58 AND L8  
L60 2 S L59 AND L9

FILE 'REGISTRY' ENTERED AT 13:41:42 ON 27 MAR 2003

L61 1 S 144378-42-7

FILE 'HCAPLUS' ENTERED AT 13:42:39 ON 27 MAR 2003

L62 6 S L61 OR PHYCOCYANIN (S) ALPHA(S)SUBUNIT (S) PHYCOCYANOBILIN LY  
L63 28 S L54-L56,L62  
L64 7 S L63 AND L12-L14  
L65 76 S HOLO? AND L8,L53-L60,L62-L64  
L66 71 S L65 AND (PD<=20010731 OR PRD<=20010731 OR AD<=20010731)  
L67 7 S L54,L55,L62 AND L66  
L68 19 S L54,L55,L62  
L69 19 S L67,L68  
L70 36 S L2-L7 AND L53-L60,L62-L63  
L71 4 S L70 AND L66  
L72 5 S L70 AND L65  
L73 5 S L71,L72  
E GENETIC ENGINEERING/CT  
E E3+ALL  
L74 79989 S E2+NT  
L75 10 S L74 AND L65  
L76 12 S L73,L75  
L77 263 S L74 AND L12-L14  
L78 8 S L77 AND L23-L29,L53-L56,L62,L63  
L79 14 S L76,L78

FILE 'HCAPLUS' ENTERED AT 13:50:25 ON 27 MAR 2003  
SEL HIT RN

FILE 'REGISTRY' ENTERED AT 13:50:40 ON 27 MAR 2003

L80 8 S E1-E8  
L81 10 S L41,L42,L43,L45-L47,L51,L52,L61 NOT L80

FILE 'HCAPLUS' ENTERED AT 13:52:50 ON 27 MAR 2003

L82 7 S L42,L52  
L83 1 S L82 NOT L79  
L84 1 S L83 AND L1-L33,L53-L60,L62-L79,L82-L83

FILE 'REGISTRY' ENTERED AT 13:54:24 ON 27 MAR 2003

L85 2 S 138263-99-7 OR 347401-20-1  
L86 2 S 114-25-0 OR 18097-67-1

FILE 'HCAPLUS' ENTERED AT 13:54:36 ON 27 MAR 2003

L87 8 S L85  
L88 753 S L86  
L89 5 S L87 AND L1-L33,L53-L60,L62-L69,L82-L84,L87,L88 NOT L79  
L90 5 S L84,L89  
L91 2 S L90 AND L88  
L92 5 S L90,L91

FILE 'HCAPLUS' ENTERED AT 13:55:52 ON 27 MAR 2003  
SEL HIT RN

FILE 'REGISTRY' ENTERED AT 13:56:10 ON 27 MAR 2003

L93 3 S E9-E15 NOT L80,L81  
SET COST ON  
SET COST OFF

FILE 'BIOSIS' ENTERED AT 13:56:44 ON 27 MAR 2003

L94           E GLAZER A/AU  
223 S E3,E5,E10,E11  
E TOOLEY A/AU  
E CAI Y/AU  
L95           190 S E3,E12  
E CAI YU/AU  
L96           7 S E3  
E CAI YUPING/AU  
L97           4 S E3  
L98           4 S E4  
E TOOLEY A/AU  
L99           2 S E4  
L100          425 S L94-L99  
L101          4 S L100 AND HOLO?  
L102          140 S L100 AND ?PHYCO?  
L103          64 S L100 AND ?BILIN?  
L104          68 S L100 AND ?BILIPROTEIN?  
L105          4 S L101 AND L102-L104

FILE 'BIOSIS' ENTERED AT 13:58:29 ON 27 MAR 2003

FILE 'BIOSIS' ENTERED AT 14:00:43 ON 27 MAR 2003

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(FILE 'HOME' ENTERED AT 12:54:49 ON 27 MAR 2003)  
SET COST OFF

FILE 'HCAPLUS' ENTERED AT 12:55:07 ON 27 MAR 2003

E US20030027285/PN  
L1 1 S E3  
E GLAZER A/AU  
L2 262 S E3,E7,E11,E13,E14  
E TOOLEY A/AU  
L3 3 S E4  
E CAI Y/AU  
L4 253 S E3-E18  
E CAI YU/AU  
L5 30 S E3,E10  
E CAI YUPING/AU  
L6 15 S E3  
L7 4 S E4  
E BILIPROTEIN/CT  
E E4+ALL  
L8 6658 S E7,E5+NT  
L9 2 S HOLOPHYCOBILIPROTEIN OR HOLO() (PHYCOBILIPROTEIN OR PHYCOBILI#

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jan.delaval@uspto.gov

FILE 'REGISTRY' ENTERED AT 13:01:53 ON 27 MAR 2003

L10 1 S 9059-22-7  
L11 1 S 14875-96-8

FILE 'HCAPLUS' ENTERED AT 13:02:57 ON 27 MAR 2003

L12 9974 S L10 OR L11  
L13 2648 S HEME OXYGENASE  
L14 30884 S HEME OR PROTOHEME OR REDUCED HEMATIN OR HEM FE OR FERROHEME O  
L15 66 S L8 AND L12-L14  
L16 2 S L9 AND L15  
L17 813 S PHYCOBILIPROTEIN  
L18 6658 S L8,L9  
L19 66 S L18 AND L12-L14  
L20 6 S L19 AND (RECOMBIN? OR CONTRUCT)  
L21 126 S L2-L7 AND L8,L9,L17,L18  
L22 4 S L12-L14 AND L21  
L23 302 S BILIN  
L24 336 S ?PHYCOCYANOBILIN?  
L25 816 S ?PHYCOBILIPROTEIN?  
L26 122 S ?PHYCOERYTHROCYANIN?  
L27 12 S ?PHYCOBILIVIOLIN?  
L28 173 S ?PHYCOERYTHROBILIN?  
L29 2074 S ?PHYCOERYTHRIN?  
L30 112 S L2-L7 AND L23-L29  
L31 4 S L12-L14 AND L30  
L32 7 S L1,L9,L16,L20,L22,L31  
L33 6 S L32 NOT PHARMACEUTICAL/TI  
SEL RN L1

FILE 'REGISTRY' ENTERED AT 13:20:58 ON 27 MAR 2003

L34 9 S E1-E9  
L35 1 S 20298-86-6  
L36 1 S 93527-36-7  
L37 45 S C33H38N4O6/MF AND NC4/ES AND 4/NR  
L38 44 S L37 AND BILIN?  
L39 7 S L38 AND 8 12 DIPROPANOIC AND 18 ETHYL 3 ETHYLIDENE  
L40 7 S L39 AND 2 7 13 17 TETRAMETHYL 1 19 DIOXO  
L41 7 S L35,L36,L40  
L42 1 S 347401-12-1

L43           E PHYCOCYANOBILIN/CN  
          1 S E8  
          E APO-PHYCOBILIPROTEIN/CN  
          E PHYCOCYANIN/CN  
L44           116 S PHYCOCYANIN (L) ALPHA (L) SUBUNIT  
L45           1 S 168680-20-4  
L46           1 S 124861-40-1  
L47           1 S 18097-67-1  
L48           174 S C33H38N4O6/MF  
L49           45 S L48 AND NC4/ES AND 4/NR  
L50           3 S L49 AND BILINE 8 12 DIPROPANOIC AND 18 ETHENYL 3 ETHYLIDENE A  
L51           3 S L47,L50  
L52           1 S 347401-21-2

FILE 'HCAPLUS' ENTERED AT 13:37:26 ON 27 MAR 2003

L53           350 S L41 OR PHYCOCYANOBILIN  
L54           8 S L42 OR PHYCOCYANOBILIN(S) FERREDOXIN(S) OXIDOREDUCTASE  
L55           8 S L45 OR PHYCOERYTHROCYANIN LYASE  
L56           12 S L46 OR PHYCOBILIVIOLIN  
L57           25 S L12-L14 AND L53  
L58           43 S L54-L57  
L59           31 S L58 AND L8  
L60           2 S L59 AND L9

FILE 'REGISTRY' ENTERED AT 13:41:42 ON 27 MAR 2003

L61           1 S 144378-42-7

FILE 'HCAPLUS' ENTERED AT 13:42:39 ON 27 MAR 2003

L62           6 S L61 OR PHYCOCYANIN (S) ALPHA(S) SUBUNIT (S) PHYCOCYANOBILIN LY  
L63           28 S L54-L56,L62  
L64           7 S L63 AND L12-L14  
L65           76 S HOLO? AND L8,L53-L60,L62-L64  
L66           71 S L65 AND (PD<=20010731 OR PRD<=20010731 OR AD<=20010731)  
L67           7 S L54,L55,L62 AND L66  
L68           19 S L54,L55,L62  
L69           19 S L67,L68  
L70           36 S L2-L7 AND L53-L60,L62-L63  
L71           4 S L70 AND L66  
L72           5 S L70 AND L65  
L73           5 S L71,L72  
          E GENETIC ENGINEERING/CT  
          E E3+ALL  
L74           79989 S E2+NT  
L75           10 S L74 AND L65  
L76           12 S L73,L75  
L77           263 S L74 AND L12-L14  
L78           8 S L77 AND L23-L29,L53-L56,L62,L63  
L79           14 S L76,L78

=> fil heaplus

FILE 'HCAPLUS' ENTERED AT 13:50:25 ON 27 MAR 2003

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FILE COVERS 1907 - 27 Mar 2003 VOL 138 ISS 13  
FILE LAST UPDATED: 26 Mar 2003 (20030326/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> d 179 all tot

L79 ANSWER 1 OF 14 HCAPLUS COPYRIGHT 2003 ACS  
AN 2003:97917 HCAPLUS  
DN 138:148684  
TI Engineering of living cells for the expression of **holo-  
phycobiliprotein**-based constructs  
IN **Glazer, Alexander N.; Tooley, Aaron J.; Cai,  
Yuping**  
PA USA  
SO U.S. Pat. Appl. Publ., 13 pp.  
CODEN: USXXCO  
DT Patent  
LA English  
IC ICM C12P021-04  
ICS C07H021-04; C12N005-06  
NCL 435069600; 435320100; 435325000; 536023200; 530380000  
CC 3-2 (Biochemical Genetics)  
Section cross-reference(s): 10, 13

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2003027285	A1	20030206	US 2001-919486	20010731 <--
	WO 2003012448	A1	20030213	WO 2002-US24245	20020730 <--
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRAI US 2001-919486 A 20010731 <--

AB Recombinant cells which express a fluorescent **holo-  
phycobiliprotein** fusion protein and methods of use are described.  
The cells comprises a **bilin**, a recombinant **bilin**  
reductase, an apo-**phycobiliprotein** fusion protein precursor of  
the fusion protein comprising a corresponding apo-**phycobiliprotein**  
domain, and a recombinant **phycobiliprotein** domain-**bilin**  
lyase, which components react to form the **holo-  
phycobiliprotein** fusion protein. Also described are **holo-  
-phycobiliprotein** based transcription reporter cells and assays,  
which cells conditionally express a heterologous-to-the-cell, fluorescent,  
first **holo-phycobiliprotein** domain.

ST living yeast bacteria mammal cell engineering **holo  
phycobiliprotein** prodn

IT **Phycocyanins**

RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BIOL  
(Biological study); PREP (Preparation); PROC (Process)  
(C-; engineering of living cells for the expression of **holo-  
phycobiliprotein**-based constructs)

- IT Enzyme functional sites  
(apo-**phycobiliprotein** domain and recombinant **phycobiliprotein** domain-**bilin** lyase; engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- IT Fluorescence resonance energy transfer  
**Genetic engineering**  
(engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- IT **Biliproteins**  
RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation); PROC (Process)  
(engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- IT Bile pigments  
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)  
(engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- IT **Phytochromes**  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- IT Fluorometry  
(for distinguishing of protein domains; engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- IT Proteins  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(green fluorescent, reporter domain of the recombinant protein; engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- IT Gene, microbial  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(hol; engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- IT Animal cell  
(mammalian, reporter cell; engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- IT Escherichia coli  
Saccharomyces cerevisiae  
(reporter cell; engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- IT Synechocystis  
(strain PCC6803; engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- IT **14875-96-8, Heme**  
RL: BCP (Biochemical process); BIOL (Biological study); PROC (Process)  
(engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- 
- IT 114-25-0P, Biliverdin 18097-67-1P, **Phycoerythrobilin** 93527-36-7P, 3(Z)-**Phycocyanobilin** 124861-40-1P, **Phycobiliviolin**  
RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation); PROC (Process)  
(engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)
- IT **347401-12-1P**  
RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation); PROC (Process)  
(gene PcyA; engineering of living cells for the expression of **holo-phycobiliprotein**-based constructs)

IT 347401-21-2P  
 RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation); PROC (Process)  
 (gene PebA and PebB; engineering of living cells for the expression of **holo-phytycobiliprotein**-based constructs)

IT 9059-22-7, **Heme oxygenase**  
 RL: BCP (Biochemical process); BIOL (Biological study); PROC (Process)  
 (gene hol, recombinant, for **bilin** formation; engineering of living cells for the expression of **holo-phytycobiliprotein**-based constructs)

IT 168680-20-4P, **Phytoerythrobilin** lyase  
 RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation); PROC (Process)  
 (heterodimeric **phytycoerythrocyanin** .alpha. subunit of, gene PecF and PecE and heterodimeric C-**phytycoerythrin** apo-.alpha. subunit domain of, gene CpeY and CpeZ; engineering of living cells for the expression of **holo-phytycobiliprotein**-based constructs)

L79 ANSWER 2 OF 14 HCAPLUS COPYRIGHT 2003 ACS

AN 2002:927638 HCAPLUS

DN 137:365572

TI Use of phytochromes for light-controlled gene expression and protein translocation into nucleus

IN Lagarius, John Clark; Kochi, Takayuki; Frankenberg, Nicole; Gambetta, Gregory A.; Montgomery, Beronda L.

PA The Regents of the University of California, USA

SO PCT Int. Appl., 102 pp.

CODEN: PIXXD2

DT Patent

LA English

IC ICM C12Q001-68

ICS C12N015-63; C12N015-85; C12N015-87; C12N015-82

CC 7-5 (Enzymes)

Section cross-reference(s): 3, 10, 11, 16

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002097137	A1	20021205	WO 2002-US17266	20020529
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
PRAI US 2001-294463P	P	20010529		

AB This invention relates to the use of heterologous phytochromes to translocate polypeptides into the nucleus of a cell. Where the polypeptides comprise transactivators or repressors this invention provides a system for light-directed gene expression. This invention identifies a novel family of **bilin** reductases. Designated herein HY2 **bilin** reductases, the enzymes of this invention are useful in a wide variety of contexts including but not limited to the conversion of biliverdins to phytobilins and the assembly of **holophytochromes** or phytofluors. The HY2 family of **bilin** reductases are ferredoxin-dependent. Using the HY2 protein sequence as a query sequence, HY2 family members were identified in the genomes of various cyanobacteria, oxyphotobacteria and plants.

ST phytochrome apoprotein light induction gene expression protein transport nucleus; HY2 **bilin** reductase cyanobacteria oxyphotobacteria

- plant; ferredoxin dependent **bilin** reductase HY2; phytyobilin  
phytofluor fermn HY2 **bilin** reductase
- IT Nuclear receptors  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(DNA binding domain of Gal4 transcription factor; use of phytochromes  
for light-controlled gene expression and protein translocation into  
nucleus)
- IT Transcription factors  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES  
(Uses)  
(GAL4, as transactivator for gene expression; use of phytochromes for  
light-controlled gene expression and protein translocation into  
nucleus)
- IT Gene  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES  
(Uses)  
(H01; cloning and use of HY2 family of ferredoxin-dependent  
**bilin** reductases from bacteria and plants)
- IT Gene, plant  
RL: BSU (Biological study, unclassified); BUU (Biological use,  
unclassified); BIOL (Biological study); USES (Uses)  
(HY2; cloning and use of HY2 family of ferredoxin-dependent  
**bilin** reductases from bacteria and plants)
- IT Transcription factors  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES  
(Uses)  
(NF-I (nuclear factor I), as transactivator for gene expression; use of  
phytochromes for light-controlled gene expression and protein  
translocation into nucleus)
- IT Transcription factors  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES  
(Uses)  
(Sp1, as transactivator for gene expression; use of phytochromes for  
light-controlled gene expression and protein translocation into  
nucleus)
- IT Transcription factors  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES  
(Uses)  
(VP16, as transactivator for gene expression; use of phytochromes for  
light-controlled gene expression and protein translocation into  
nucleus)
- IT Proteins  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES  
(Uses)  
(apoproteins, phytochrome; use of phytochromes for light-controlled  
gene expression and protein translocation into nucleus)
- IT Anabaena  
Arabidopsis thaliana  
Barley  
Cyanobacteria  
Embryophyta  
Fermentation  
Fluorescent indicators  
**Molecular cloning**  
Plastid  
Prochlorococcus marinus  
Synechococcus  
Synechocystis  
(cloning and use of HY2 family of ferredoxin-dependent **bilin**  
reductases from bacteria and plants)
- IT Fusion proteins (chimeric proteins)  
RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BIOL  
(Biological study); PREP (Preparation); PROC (Process)

- (cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT **Phytochromes**  
 RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)  
 (cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT Transcription factors  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (lactose repressors, for gene expression; use of phytochromes for light-controlled gene expression and protein translocation into nucleus)
- IT Proteins  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (linker, peptide; use of phytochromes for light-controlled gene expression and protein translocation into nucleus)
- IT Evolution  
 (mol., of HY2 family of **bilin** reductases; cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (pcyA; cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT Operon  
 (peb; cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (pebA; cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (pebB; cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT Bile pigments  
 RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)  
 (phytyobilins; cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT Bile pigments  
 RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)  
 (phytofluors; cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- 
- IT Cytoplasm  
 (protein targeting to nucleus from; use of phytochromes for light-controlled gene expression and protein translocation into nucleus)
- IT Transcription factors  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (repressors, lex, for gene expression; use of phytochromes for light-controlled gene expression and protein translocation into nucleus)
- IT Transcription factors  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(repressors, tet, for gene expression; use of phytochromes for light-controlled gene expression and protein translocation into nucleus)

- IT Biological transport  
Cell nucleus  
Genome  
Light  
(use of phytochromes for light-controlled gene expression and protein translocation into nucleus)
- IT Proteins  
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(use of phytochromes for light-controlled gene expression and protein translocation into nucleus)
- IT 114-25-0, Biliverdin 78249-71-5, Phytochromobilin  
RL: BCP (Biochemical process); BIOL (Biological study); PROC (Process)  
(cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT **9059-22-7P, Heme oxygenase**  
RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation); PROC (Process)  
(cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT 160047-82-5P, Biliverdin IX.alpha. reductase 199618-44-5P  
RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)  
(cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT 138263-99-7P  
RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)  
(gene HY2; cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT **347401-12-1P**  
RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)  
(gene pcyA; cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT 347401-20-1P  
RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)  
(gene pebA; cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT 347401-21-2P  
RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)  
(gene pebB; cloning and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

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RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD  
RE

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- (3) Quail; US 5656496 A 1997 HCAPLUS
- (4) Summers; US 6017734 A 2000 HCAPLUS

L79 ANSWER 3 OF 14 HCAPLUS COPYRIGHT 2003 ACS  
AN 2002:629384 HCAPLUS  
DN 138:181625

- TI Biosynthesis of the cyanobacterial light-harvesting polypeptide **phycoerythrocyanin holo**-.alpha. subunit in a heterologous host
- AU **Tooley, Aaron J.; Glazer, Alexander N.**
- CS Department of Molecular and Cell Biology, University of California, Berkeley, CA, 94720-3200, USA
- SO Journal of Bacteriology (2002), 184(17), 4666-4671  
CODEN: JOBAA; ISSN: 0021-9193
- PB American Society for Microbiology
- DT Journal
- LA English
- CC 3-2 (Biochemical Genetics)  
Section cross-reference(s): 6, 10
- AB The entire pathway for the biosynthesis of the **phycobiliviolin**-bearing His-tagged **holo**-.alpha. subunit of the cyanobacterial photosynthetic accessory protein **phycoerythrocyanin** was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of **heme** to 3Z-**phycocyanobilin**, a precursor of **phycobiliviolin** (namely, **heme oxygenase 1** and 3Z-**phycocyanobilin: ferredoxin oxidoreductase**), were expressed from a plasmid under the control of the hybrid *trp-lac* (*trc*) promoter. Genes for the apo-**phycoerythrocyanin** .alpha. subunit (*pecA*) and the heterodimeric lyase/isomerase (*pecE* and *pecF*), which catalyzes both the covalent attachment of **phycocyanobilin** and its concurrent isomerization to **phycobiliviolin**, were expressed from the *trc* promoter on a second plasmid. Upon induction, recombinant *E. coli* used endogenous **heme** to produce **holo**-PecA with absorbance and fluorescence properties similar to those of the same protein produced in cyanobacteria. About two-thirds of the apo-PecA was converted to **holo**-PecA. No significant **bilin** addn. took place in a similarly engineered *E. coli* strain that lacks *pecE* and *pecF*. By using immobilized metal affinity chromatog., both apo-PecA and **holo**-PecA were isolated as ternary complexes with PecE and PecF. The identities of all three components in the ternary complexes were established unambiguously by protein and tryptic peptide analyses performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.
- ST *Anabaena* cyanobacteria **phycoerythrocyanin pecA holo** alpha subunit *Escherichia*
- IT **Genetic engineering**  
(Biosynthesis of the cyanobacterial light-harvesting polypeptide **phycoerythrocyanin holo**-.alpha. subunit in a heterologous host)
- IT *Anabaena*  
*Escherichia coli*  
(biosynthesis of *Anabaena* light-harvesting polypeptide **phycoerythrocyanin holo**-.alpha. subunit in *Escherichia coli*)
- 
- IT Gene, microbial  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(*pecA*; biosynthesis of *Anabaena* light-harvesting polypeptide **phycoerythrocyanin holo**-.alpha. subunit in *Escherichia coli*)
- IT Gene, microbial  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(*pecE*; biosynthesis of *Anabaena* light-harvesting polypeptide **phycoerythrocyanin holo**-.alpha. subunit in *Escherichia coli*)
- IT Gene, microbial  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(*pecF*; biosynthesis of *Anabaena* light-harvesting polypeptide **phycoerythrocyanin holo**-.alpha. subunit in

*had date*

- Escherichia coli)
- IT **Phycocyanins**  
**Phycoerythrins**  
 RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified);  
 BIOL (Biological study); PREP (Preparation)  
 (phycoerythrocyanins, .alpha. subunit, complex with PecE and  
 PecF; biosynthesis of Anabaena light-harvesting polypeptide  
 phycoerythrocyanin holo-.alpha. subunit in  
 Escherichia coli)
- IT **9059-22-7**  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (1, gene for; biosynthesis of Anabaena light-harvesting polypeptide  
 phycoerythrocyanin holo-.alpha. subunit in  
 Escherichia coli in relation to)
- IT **14875-96-8, Heme 20298-86-6,**  
**Phycocyanobilin 124861-40-1, Phycobiliviolin**  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (biosynthesis of Anabaena light-harvesting polypeptide  
 phycoerythrocyanin holo-.alpha. subunit in  
 Escherichia coli)
- IT **347401-12-1, 3Z-Phycocyanobilin:ferredoxin**  
**oxidoreductase**  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (gene for; biosynthesis of Anabaena light-harvesting polypeptide  
 phycoerythrocyanin holo-.alpha. subunit in  
 Escherichia coli in relation to)
- IT **168680-20-4, Phycoerythrocyanin .alpha. subunit lyase**  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (genes pecE and pecF for; biosynthesis of Anabaena light-harvesting  
 polypeptide phycoerythrocyanin holo-.alpha. subunit  
 in Escherichia coli)

RE.CNT 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD  
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L79 ANSWER 4 OF 14 HCAPLUS COPYRIGHT 2003 ACS

AN 2001:904470 HCAPLUS

DN 136:50278

TI Identification, cloning, sequences and use of HY2 family of  
 ferredoxin-dependent bilin reductases from bacteria and plants

IN Lagarias, John Clark; Rochi, Takayuki; Frankenberg, Nicole; Gambetta,

Gregory A.; Montgomery, Beronda L.  
 PA The Regents of the University of California, USA  
 SO PCT Int. Appl., 102 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 ICI C12  
 CC 7-5 (Enzymes)

Section cross-reference(s): 3, 10, 11, 16

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001094548	A2	20011213	WO 2001-US18326	20010605
	WO 2001094548	A3	20020711		
	W: CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
	EP 1290135	A2	20030312	EP 2001-942007	20010605
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY, TR				

PRAI US 2000-210286P P 20000608  
 WO 2001-US18326 W 20010605

AB This invention identifies a novel family of **bilin** reductases. Designated herein HY2 **bilin** reductases, the enzymes of this invention are useful in a wide variety of contexts including but not limited to the conversion of biliverdins to phytobilins and the assembly of **holophytochromes** or phytofluors. The HY2 family of **bilin** reductases are ferredoxin-dependent. The genomic sequence and the encoded protein sequence of the gene HY2 phytochromobilin synthase of Arabidopsis thaliana are disclosed. Using the HY2 protein sequence as a query sequence, HY2 family members were identified in the genomes of various cyanobacteria, oxyphotobacteria and plants.

ST HY2 **bilin** reductase cyanobacteria oxyphotobacteria plant sequence; ferredoxin dependent **bilin** reductase HY2 sequence; phytobilin phytofluor fermn HY2 **bilin** reductase

IT Gene  
 RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
 (H01; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT Gene, plant  
 RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
 (HY2; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT Bacteria (Eubacteria)  
 Insecta

Plant cell

Yeast

(cloning host; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT Algae  
 Anabaena  
 Arabidopsis thaliana  
 Barley  
 Cyanobacteria  
 DNA sequences  
 Embryophyta  
 Fermentation  
 Fluorescent indicators

**Molecular cloning**

Nostoc punctiforme

Oxyphotobacteria

Plastid

Prochlorococcus

Protein sequences

Synechococcus

Synechocystis

(identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT Fusion proteins (chimeric proteins)

RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation); PROC (Process)

(identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT **Phytochromes**

RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)

(identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT Animal cell

(mammalian, cloning host; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT Evolution

(mol.; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT Gene, microbial

RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses) (pcyA; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT Operon

(peb; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT Gene, microbial

RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses) (pebA; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT Gene, microbial

RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses) (pebB; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT Bile pigments

RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)

(phytyobilins; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT Bile pigments

RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)

(phytofluors; identification, cloning, sequences and use of HY2 family

- of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT 381665-56-1P 381665-57-2P 381665-58-3P 381665-59-4P 381665-60-7P  
 381665-84-5P 381665-85-6P 381665-86-7P 381665-87-8P 381665-88-9P  
 381665-89-0P 381665-90-3P 381665-91-4P 381665-92-5P 381665-93-6P  
 381665-94-7P 381665-95-8P 381665-96-9P 381665-97-0P 381665-98-1P  
 381665-99-2P 381666-00-8P 381741-62-4P  
 RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); PROC (Process)  
 (amino acid sequence; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT 138263-99-7P  
 RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); PROC (Process)  
 (gene HY2; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT 347401-12-1P  
 RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); PROC (Process)  
 (gene pcyA; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT 347401-20-1P  
 RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); PROC (Process)  
 (gene pebA; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT 347401-21-2P  
 RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); PROC (Process)  
 (gene pebB; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT 114-25-0, Biliverdin 78249-71-5, Phytochromobilin  
 RL: BCP (Biochemical process); BIOL (Biological study); PROC (Process)  
 (identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT 9059-22-7P, Heme oxygenase  
 RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation); PROC (Process)  
 (identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- 
- IT 160047-82-5P, Biliverdin IX.alpha. reductase 199618-44-5P  
 RL: BCP (Biochemical process); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); PROC (Process)  
 (identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)
- IT 381741-61-3  
 RL: BCP (Biochemical process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process); USES (Uses)

(nucleotide sequence; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

IT	381747-43-9	381747-44-0	381747-45-1	381747-46-2	381747-47-3
	381747-48-4	381747-49-5	381747-50-8	381747-51-9	381747-52-0
	381747-53-1	381747-54-2	381747-55-3	381747-56-4	381747-57-5
	381747-58-6	381747-59-7	381747-60-0	381747-61-1	381747-62-2
	381747-63-3	381747-64-4	381747-65-5	381747-66-6	381747-67-7
	381747-68-8	381747-69-9	381747-70-2	381747-71-3	381747-72-4
	381747-74-6				

RL: PRP (Properties)

(unclaimed nucleotide sequence; identification, cloning, sequences and use of HY2 family of ferredoxin-dependent **bilin** reductases from bacteria and plants)

L79 ANSWER 5 OF 14 HCAPLUS COPYRIGHT 2003 ACS

AN 2001:866304 HCAPLUS

DN 136:180441

TI Recombinant **holophytochrome** in Escherichia coli

AU Landgraf, F. T.; Forreiter, C.; Hurtado Pico, A.; Lamparter, T.; Hughes, J.

CS Plant Physiology, Daz Zeughaus, Justus-Liebig-University Giessen, Giessen, D-35390, Germany

SO FEBS Letters (2001), 508(3), 459-462

CODEN: FEBLAL; ISSN: 0014-5793

PB Elsevier Science B.V.

DT Journal

LA English

CC 10-4 (Microbial, Algal, and Fungal Biochemistry)

AB We have successfully co-expressed two genes from the **bilin** biosynthetic pathway of Synechocystis together with cyanobacterial phytochrome 1 (Cph1) from the same organism to produce **holophytochrome** in Escherichia coli. **Heme oxygenase** was used to convert host **heme** to biliverdin IX.alpha. which was then reduced to **phycocyanobilin** via **phycocyanobilin:ferredoxin oxidoreductase**, presumably with the aid of host **ferredoxin**. In this host environment Cph1 apophytochrome was able to autoassemble with the **phycocyanobilin** in vivo to form fully photoreversible **holophytochrome**. The system can be used as a tool for further genetic studies of phytochrome function and signal transduction as well as providing an excellent source of **holophytochrome** for physicochem. studies.

ST Escherichia recombinant **holophytochrome** formation

IT **Phytochromes**

RL: BSU (Biological study, unclassified); BIOL (Biological study) (Phytochrome 1, apo- and **holo**-; recombinant **holophytochrome** in Escherichia coli)

IT Gene, microbial

RL: BSU (Biological study, unclassified); BIOL (Biological study) (cph1; recombinant **holophytochrome** in Escherichia coli)

IT Gene, microbial

RL: BSU (Biological study, unclassified); BIOL (Biological study) (hol; recombinant **holophytochrome** in Escherichia coli)

IT Gene, microbial

RL: BSU (Biological study, unclassified); BIOL (Biological study) (pcyA; recombinant **holophytochrome** in Escherichia coli)

IT **Molecular cloning**

Synechocystis

(recombinant **holophytochrome** in Escherichia coli)

IT Ferredoxins

RL: BSU (Biological study, unclassified); BIOL (Biological study) (recombinant **holophytochrome** in Escherichia coli)

IT Escherichia coli  
(recombinant; recombinant **holophytochrome** in Escherichia coli)

IT 114-25-0, Biliverdin IX.alpha. 9059-22-7, **Heme oxygenase** 14875-96-8, **Heme** 20298-86-6, **Phycocyanobilin** 347401-12-1  
RL: BSU (Biological study, unclassified); BIOL (Biological study) (recombinant **holophytochrome** in Escherichia coli)

RE.CNT 17 . THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L79 ANSWER 6 OF 14 HCAPLUS COPYRIGHT 2003 ACS

AN 2001:705482 HCAPLUS

DN 135:369332

TI Genetic engineering of phytochrome biosynthesis in bacteria

AU Gambetta, Gregory A.; Lagarias, J. Clark

CS Section of Molecular and Cellular Biology, University of California, Davis, CA, 95616, USA

SO Proceedings of the National Academy of Sciences of the United States of America (2001), 98(19), 10566-10571  
CODEN: PNASA6; ISSN: 0027-8424

PB National Academy of Sciences

DT Journal

LA English

CC 11-8 (Plant Biochemistry)  
Section cross-reference(s): 3

AB The **bilin** prosthetic groups of the phytochrome photoreceptors and the light-harvesting **phycobiliprotein** antennae arise from the oxygen-dependent ring opening of **heme**. Two ferredoxin-dependent enzymes contribute to this conversion: a **heme oxygenase** and a **bilin** reductase with discrete double-bond specificity. Using a dual plasmid system, one expressing a truncated cyanobacterial apophytochrome 1, Cph1(N514), and the other expressing a two-gene operon consisting of a **heme oxygenase** and a **bilin** reductase, these studies establish the feasibility of producing photoactive phytochromes in any **heme**-contg. cell. Heterologous expression systems for phytochromes not only will facilitate genetic anal. of their assembly, spectrophotometric activity, and biol. function, but also might afford the means to regulate gene expression by light in nonplant cells.

ST Escherichia phytochrome biosynthesis

IT Proteins, specific or class  
RL: BPN (Biosynthetic preparation); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)  
(Cph1(N514); genetic engineering of phytochrome biosynthesis in bacteria)

IT Promoter (genetic element)  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (ara; genetic engineering of phytochrome biosynthesis in bacteria)

IT Gene, microbial  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (cph1; genetic engineering of phytochrome biosynthesis in bacteria)

IT **Molecular cloning**  
 (genetic engineering of phytochrome biosynthesis in bacteria)

IT Phytochromes  
 RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)  
 (genetic engineering of phytochrome biosynthesis in bacteria)

IT Gene, microbial  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (hol; genetic engineering of phytochrome biosynthesis in bacteria)

IT Gene, microbial  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (pcyA; genetic engineering of phytochrome biosynthesis in bacteria)

IT Escherichia coli  
 (recombrnant; genetic engineering of phytochrome biosynthesis in bacteria)

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L79 ANSWER 7 OF 14 HCAPLUS COPYRIGHT 2003 ACS  
 AN 2001:705481 HCAPLUS  
 DN 136:2664  
 TI Biosynthesis of a fluorescent cyanobacterial C-phyocyanin **holo**  
 -.alpha. subunit in a heterologous host  
 AU **Tooley, Aaron J.; Cai, Yuping A.; Glazer, Alexander N.**  
 CS Department of Molecular and Cell Biology, University of California,  
 Berkeley, CA, 94720-3200, USA  
 SO Proceedings of the National Academy of Sciences of the United States of  
 America (2001), 98(19), 10560-10565  
 CODEN: PNASA6; ISSN: 0027-8424  
 PB National Academy of Sciences  
 DT Journal  
 LA English  
 CC 10-2 (Microbial, Algal, and Fungal Biochemistry)  
 Section cross-reference(s): 3  
 AB The entire pathway for the synthesis of a fluorescent  
**holophycobiliprotein** subunit from a photosynthetic cyanobacterium  
 (Synechocystis sp. PCC6803) was reconstituted in Escherichia coli.  
 Cyanobacterial genes encoding enzymes required for the conversion of  
**heme** to the natural chromophore 3Z-**phyocyanobilin**,  
 namely, **heme oxygenase 1** and 3Z-  
**phyocyanobilin:ferredoxin oxidoreductase**,  
 were expressed from a plasmid under control of the hybrid trp-lac (trc)  
 promoter. Genes for the apoprotein (C-phyocyanin .alpha. subunit; cpcA)  
 and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore  
 attachment were expressed from the trc promoter on a second plasmid. Upon  
 induction, recombinant E. coli used the cellular pool of **heme** to  
 produce **holo-CpcA** with spectroscopic properties qual. and quant.  
 similar to those of the same protein produced endogenously in  
 cyanobacteria. About a third of the apo-CpcA was converted to  
**holo-CpcA**. No significant **bilin** addn. took place in a  
 similarly engineered E. coli strain that lacks cpcE and cpcF. This  
 approach should permit incisive anal. of many remaining questions in  
**phycobiliprotein** biosynthesis. These studies also demonstrate the  
 feasibility of generating constructs of these proteins in situ for use as  
 fluorescent protein probes in living cells.  
 ST Synechocystis phyocyanin formation  
 IT **Phyocyanins**  
 RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL  
 (Biological study); FORM (Formation, nonpreparative)  
 (C-; biosynthesis of fluorescent cyanobacterial C-phyocyanin  
**holo**-.alpha. subunit in heterologous host)  
 IT Escherichia coli  
**Molecular cloning**  
 Synechocystis  
 (biosynthesis of fluorescent cyanobacterial C-phyocyanin **holo**  
 -.alpha. subunit in heterologous host)  


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 IT 144378-42-7P, **Phyocyanin .alpha.-**  
**subunit phyocyanobilin lyase**  
 347401-12-1P, **Ferredoxin:3Z-phyocyanobilin**  
**oxidoreductase**  
 RL: BAC (Biological activity or effector, except adverse); BPN  
 (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL  
 (Biological study); PREP (Preparation)  
 (biosynthesis of fluorescent cyanobacterial C-**phyocyanin**  
**holo**-.alpha. subunit in heterologous host)  
 IT 14875-96-8, **Heme**  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL  
 (Biological study); PROC (Process)  
 (biosynthesis of fluorescent cyanobacterial C-phyocyanin **holo**

- .alpha. subunit in heterologous host)
- IT 93527-36-7  
 RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process)  
 (biosynthesis of fluorescent cyanobacterial C-phyococyanin **holo**-.alpha. subunit in heterologous host)
- IT 9059-22-7P, **Heme oxygenase**  
 RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
 (isoform 1; biosynthesis of fluorescent cyanobacterial C-phyococyanin **holo**-.alpha. subunit in heterologous host)

RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD  
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L79 ANSWER 8 OF 14 HCAPLUS COPYRIGHT 2003 ACS

AN 2001:465506 HCAPLUS

DN 135:192925

TI ~~The heme-oxygenase family required for phytochrome chromophore~~  
 biosynthesis is necessary for proper photomorphogenesis in higher plants

AU Davis, Seth J.; Bhoo, Seong Hee; Durski, Adam M.; Walker, Joseph M.;  
 Vierstra, Richard D.

CS Laboratory of Genetics, Cellular and Molecular Biology Program, University  
 of Wisconsin, Madison, WI, 53706, USA

SO Plant Physiology (2001), 126(2), 656-669  
 CODEN: PLPHAY; ISSN: 0032-0889

PB American Society of Plant Physiologists

DT Journal

LA English

CC 11-3 (Plant Biochemistry)

Section cross-reference(s): 3

AB The committed step in the biosynthesis of the phytochrome chromophore

phytochromobilin involves the oxidative cleavage of heme by a heme oxygenase (HO) to form biliverdin IX.alpha.. Through positional cloning of the photomorphogenic mutant hyl, the Arabidopsis HO (designated AtH01) responsible for much of phytochromobilin synthesis recently was identified. Using the AtH01 sequence, we identified families of HO genes in a no. of plants that cluster into two subfamilies (H01- and H02-like). The tomato (*Lycopersicon esculentum*) yg-2 and *Nicotiana plumbaginifolia* pew1 photomorphogenic mutants are defective in specific HO genes. Phenotypic anal. of a T-DNA insertion mutant of Arabidopsis H02 revealed that the second HO subfamily also contributes to phytochromobilin synthesis. Homozygous ho2-1 plants show decreased chlorophyll accumulation, reduced growth rate, accelerated flowering time, and reduced de-etiolation. A mixt. of apo- and **holo**-phyA was detected in etiolated ho2-1 seedlings, suggesting that phytochromobilin is limiting in this mutant, even in the presence of functional AtH01. The patterns of Arabidopsis H01 and H02 expression suggest that the products of both genes overlap temporally and spatially. Taken together, the family of HOs is important for phytochrome-mediated development in a no. of plants and that each family member may uniquely contribute to the phytochromobilin pool needed to assemble **holo**-phytochromes.

ST Arabidopsis HO gene heme oxygenase phytochrome mol cloning

IT Gene, plant

RL: BOC (Biological occurrence); BSU (Biological study, unclassified);  
BIOL (Biological study); OCCU (Occurrence)  
(athol; heme-oxygenase family is necessary for proper  
photomorphogenesis in higher plants)

IT Gene, plant

RL: BOC (Biological occurrence); BSU (Biological study, unclassified);  
BIOL (Biological study); OCCU (Occurrence)  
(atho2; heme-oxygenase family is necessary for proper  
photomorphogenesis in higher plants)

IT Arabidopsis thaliana

#### **Molecular cloning**

Tobacco (*Nicotiana plumbaginifolia*)

Tomato

Transformation, genetic

(heme-oxygenase family is necessary for proper photomorphogenesis in  
higher plants)

IT **Phytochromes**

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL  
(Biological study); PROC (Process)  
(heme-oxygenase family is necessary for proper photomorphogenesis in  
higher plants)

IT Protein sequences

(of heme oxygenase; heme-oxygenase family is necessary for proper  
photomorphogenesis in higher plants)

IT Growth and development, plant

(photomorphogenesis; heme-oxygenase family is necessary for proper  
photomorphogenesis in higher plants)

IT Proteins, specific or class

RL: BOC (Biological occurrence); BSU (Biological study, unclassified);  
BIOL (Biological study); OCCU (Occurrence)  
(phy; heme-oxygenase family is necessary for proper photomorphogenesis  
in higher plants)

IT 9059-22-7, Heme oxygenase

RL: BAC (Biological activity or effector, except adverse); BSU (Biological  
study, unclassified); PRP (Properties); BIOL (Biological study)  
(heme-oxygenase family is necessary for proper photomorphogenesis in  
higher plants)

RE.CNT 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

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L79 ANSWER 9 OF 14 HCAPLUS COPYRIGHT 2003 ACS

AN 2000:584024 HCAPLUS

DN 134:25870

TI Phytobilin biosynthesis: the *Synechocystis* sp. PCC 6803 **heme oxygenase**-encoding *hol* gene complements a phytochrome-deficient *Arabidopsis thaliana* *hyl* mutant

AU Willows, Robert D.; Mayer, Sandra M.; Foulk, Michael S.; DeLong, Alison; Hanson, Kimberly; Chory, Joanne; Beale, Samuel I.

CS Division of Biology and Medicine, Brown University, Providence, RI, 02912, USA

SO Plant Molecular Biology (2000), 43(1), 113-120  
CODEN: PMBIDB; ISSN: 0167-4412

PB Kluwer Academic Publishers

- DT Journal  
 LA English  
 CC 3-2 (Biochemical Genetics)  
 Section cross-reference(s): 7, 11
- AB The phytyobilin chromophores of **phycobiliproteins** and phytochromes are biosynthesized from **heme** in a pathway that begins with the opening of the tetrapyrrole macrocycle of **protoheme** to form biliverdin IX.alpha., in a reaction catalyzed by **heme oxygenase**. An Arabidopsis thaliana hyl mutant was previously shown to be deficient in phytochrome responses, and these responses were regained when the plants were administered biliverdin IX.alpha.. A **heme oxygenase**-encoding gene, hol, was recently cloned from the cyanobacterium Synechocystis sp. PCC 6083. When hol was expressed in Escherichia coli, the cells produced active ferredoxin-dependent sol. **heme oxygenase**. The open reading frame of hol was fused in frame with a chloroplast transit peptide-encoding sequence from the oli gene of Antirrhinum majus. This construct was placed in a binary plasmid vector contg. a kanamycin resistance marker and a cauliflower mosaic virus 35S promoter to control expression of the chimeric oli-hol gene and used to transform A. thaliana hyl plants. Two independent transformed lines were obtained that had the phenotype of the parental Landsberg erecta line and expressed the chimeric gene, as indicated by detection of its mRNA by reverse transcriptase-polymerase chain reaction. The results indicate that Synechocystis sp. PCC 6803 **heme oxygenase** encoded by hol can substitute for the defective HY1 gene product and that the only required enzyme activity of the HY1 gene product is **heme oxygenase**.
- ST Synechocystis gene hol **heme oxygenase** complement phytochrome deficient Arabidopsis; cloning Synechocystis **heme oxygenase** complement phytochrome deficient Arabidopsis hyl
- IT Complementation (genetic)  
**Molecular cloning**  
 (Synechocystis sp. PCC 6803 **heme oxygenase**-encoding hol gene complements phytochrome-deficient Arabidopsis thaliana hyl mutant)
- IT Phytochromes  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (Synechocystis sp. PCC 6803 **heme oxygenase**-encoding hol gene complements phytochrome-deficient Arabidopsis thaliana hyl mutant)
- IT Snapdragon (Antirrhinum majus)  
 (chimeric oli-hol gene, which contains chloroplast transit peptide-encoding sequence of Antirrhinum majus oli gene fused to Synechocystis PCC 6803 hol gene, used to transform phytochrome-deficient Arabidopsis thaliana hyl mutant)
- IT Gene, microbial  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (hol; Synechocystis sp. PCC 6803 **heme oxygenase**-encoding hol gene complements phytochrome-deficient Arabidopsis thaliana hyl mutant)
- IT Chimeric gene  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (oli-hol; chimeric oli-hol gene, which contains chloroplast transit peptide-encoding sequence of Antirrhinum majus oli gene fused to Synechocystis PCC 6803 hol gene, used to transform phytochrome-deficient Arabidopsis thaliana hyl mutant)
- IT Gene, plant  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (oli; chimeric oli-hol gene, which contains chloroplast transit

peptide-encoding sequence of Antirrhinum majus oli gene fused to Synechocystis PCC 6803 hol gene, used to transform phytochrome-deficient Arabidopsis thaliana hyl mutant)

- IT Protein sequences  
(protein sequence comparison of Arabidopsis thaliana HY1 protein, Synechocystis PCC 6803 **heme oxygenase** and human **heme oxygenase 1**)
- IT Synechocystis  
(sp. PCC 6803; Synechocystis sp. PCC 6803 **heme oxygenase**-encoding hol gene complements phytochrome-deficient Arabidopsis thaliana hyl mutant)
- IT Arabidopsis thaliana  
(transformed; Synechocystis sp. PCC 6803 **heme oxygenase**-encoding hol gene complements phytochrome-deficient Arabidopsis thaliana hyl mutant)
- IT 9059-22-7P, **Heme oxygenase**  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(Synechocystis sp. PCC 6803 **heme oxygenase**-encoding hol gene complements phytochrome-deficient Arabidopsis thaliana hyl mutant)

RE.CNT 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD  
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L79 ANSWER 10 OF 14 HCAPLUS COPYRIGHT 2003 ACS

AN 1996:524194 HCAPLUS

DN 125:190274

TI The methylotrophic yeast Pichia pastoris synthesizes a functionally active chromophore precursor of the plant photoreceptor phytochrome

AU Wu, Shu-Hsing; Lagarias, J. Clark  
 CS Section Molecular Cellular Biology, Univ. California, Davis, CA, 95616, USA  
 SO Proceedings of the National Academy of Sciences of the United States of America (1996), 93(17), 8989-8994  
 CODEN: PNASA6; ISSN: 0027-8424  
 PB National Academy of Sciences  
 DT Journal  
 LA English  
 CC 10-2 (Microbial, Algal, and Fungal Biochemistry)  
 Section cross-reference(s): 3  
 AB Induction of the expression of an algal phytochrome cDNA in the methylotrophic yeast *Pichia pastoris* led to time-dependent formation of photoactive **holophytochrome** without the addn. of exogenous bilins. Both in vivo and in vitro difference spectra of this photochromic species are very similar to those of higher plant phytochrome A, supporting the conclusion that this species possesses a phytochromobilin prosthetic group. Zinc blot analyses confirm that a bilin chromophore is covalently bound to the algal phytochrome apoprotein. The hypothesis that *P. pastoris* contains phytochromobilin synthase, the enzyme that converts biliverdin IX.alpha. to phytochromobilin, was also addressed in this study. Sol. exts. from *P. pastoris* were able to convert biliverdin to a bilin pigment, which produced a native difference spectrum upon assembly with oat apophytochrome A. HPLC analyses confirm that biliverdin is converted to both 3E- and 3Z-isomers of phytochromobilin. These investigations demonstrate that the ability to synthesize phytochromobilin is not restricted to photosynthetic organisms and support the hypothesis of a more widespread distribution of the phytochrome photoreceptor.

ST phytochrome *Pichia*  
 IT **Molecular cloning**  
*Pichia pastoris*  
 (methylotrophic yeast *Pichia pastoris* synthesizes functionally active chromophore precursor of plant photoreceptor phytochrome)

IT **Phytochromes**  
 RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)  
 (methylotrophic yeast *Pichia pastoris* synthesizes functionally active chromophore precursor of plant photoreceptor phytochrome)

IT 138263-99-7, Phytochromobilin synthase  
 RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)  
 (*Pichia pastoris* phytochromobilin synthase in relation to phytochrome formation)

IT 114-25-0, Biliverdin  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (biliverdin metab. in *Pichia pastoris*)

IT 78249-71-5, Phytochromobilin  
 RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)  
 (from biliverdin metab. in *Pichia pastoris*)

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L79 ANSWER 11 OF 14 HCAPLUS COPYRIGHT 2003 ACS  
 AN 1995:597221 HCAPLUS  
 DN 123:250826  
 TI Candidate genes for the phycoerythrocyanin .alpha. subunit lyase and biochemical analysis of pecE and pecF interposon mutants  
 AU Jung, Linda J.; Chan, Crystal F.; Glazer, Alexander N.  
 CS Department Molecular Cell Biology, University California, Berkeley, CA, 94720, USA  
 SO Journal of Biological Chemistry (1995), 270(21), 12877-84  
 CODEN: JBCHA3; ISSN: 0021-9258

PB American Society for Biochemistry and Molecular Biology  
 DT Journal  
 LA English  
 CC 10-1 (Microbial, Algal, and Fungal Biochemistry)  
 Section cross-reference(s): 3, 6  
 AB The rod substructures of the *Anabaena* sp. PCC 7120 phycobilisome contain the light harvesting proteins C-phyococyanin and phycoerythrocyanin (PEC). Even at low light intensities, PEC represents no more than 5% of the phycobilisome protein. The .beta. subunits of both proteins carry thioether-linked **phycocyanobilin** (PCB) at .beta.-Cys-82 and .beta.-Cys-155; however, C-phyococyanin has PCB at .alpha.-Cys-84 whereas PEC .alpha. subunit carries **phycobiliviolin** at this position. The *Anabaena* sp. PCC 7120 pec operon is made up of five genes. PecB and pecA encode the .beta. and .alpha. subunits of PEC, pecC encodes a linker polypeptide assocd. with PEC in the rod substructure, and pecE and pecF are genes of unknown function that show a high degree of homol. to cpcE and cpcF, that encode a C-phyococyanin .alpha. subunit PCB lyase (Fairchild, C. D., Zhao, J., Zhou, J., Colson, S. E., Bryant, D. A., and Glazer, A. N. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 7017-7021). Insertional mutants in pecE and pecF, and an interposon mutant in which a portion of both pecE and pecF was deleted, were constructed. All three types of mutants grew 1.3 times slower than wild-type under limiting light conditions and showed a 20% redn. in the PCB content of whole cells relative to chlorophyll a. **Holo-PEC** was missing from the phycobilisomes of all three types of mutants and the level of the PEC linker polypeptide was reduced relative to the PEC linker polypeptide was reduced relative to the wild-type. However, .apprx.30% of the wild-type level of the PEC .beta. subunit was present in all of these phycobilisomes. In contrast, the PEC .alpha. subunit was barely detectable in the pecE and pecF mutants, but was present in the pecEF deletion mutant as a PCB-adduct in a 1:1 ratio with the PEC .beta. subunit. The identity of this "unnatural" adduct was confirmed by isolation of the subunit and amino-terminal sequencing. These biochem. results support the inference that pecE and pecF encode a PEC .alpha. subunit **phycobiliviolin** lyase, and, in conjunction with earlier findings, demonstrate that phycobiliprotein bilin lyases show high selectivity (rather than abs. specificity) for both the bilin and the polypeptide substrate.

ST phycoerythrocyanin alpha subunit lyase pecE pecF; *Anabaena*  
 phycoerythrocyanin subunit lyase gene pec  
 IT *Anabaena*  
 Phycobilisome  
 (candidate genes for phycoerythrocyanin .alpha. subunit lyase and  
 biochem. anal. of pecE and pecF interposon mutants)  
 IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (pecE; candidate genes for phycoerythrocyanin .alpha. subunit lyase and  
 biochem. anal. of pecE and pecF interposon mutants)  
 IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (pecF; candidate genes for phycoerythrocyanin .alpha. subunit lyase and  
 biochem. anal. of pecE and pecF interposon mutants)  
 IT **Biliproteins**  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (**phycoerythrocyanins**, candidate genes for phycoerythrocyanin  
 .alpha. subunit lyase and biochem. anal. of pecE and pecF interposon  
 mutants)  
 IT **168680-20-4**, Phycoerythrocyanin .alpha.-subunit lyase  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (candidate genes for phycoerythrocyanin .alpha. subunit lyase and  
 biochem. anal. of pecE and pecF interposon mutants)

AN 1992:628825 HCAPLUS  
 DN 117:228825  
 TI **Phycocyanin .alpha.-subunit  
 phycocyanobilin lyase**  
 AU Fairchild, Craig D.; Zhao, Jindong; Zhou, Jianhui; Colson, Sue Ellen;  
 Bryant, Donald A.; **Glazer, Alexander N.**  
 CS Dep. Mol. Cell Biol., Univ. California, Berkeley, CA, 94720, USA  
 SO Proceedings of the National Academy of Sciences of the United States of  
 America (1992), 89(15), 7017-21  
 CODEN: PNASA6; ISSN: 0027-8424  
 DT Journal  
 LA English  
 CC 7-2 (Enzymes)  
 Section cross-reference(s): 3  
 AB Phycobiliproteins, unlike other light-harvesting proteins involved in  
 photosynthesis, bear covalently attached chromophores. The bilin  
 chromophores are attached through thioether bonds to cysteine residues.  
 The cyanobacterium *Synechococcus* sp. PCC7002 has eight distinct bilin  
 attachment sites on seven polypeptides, all of which carry the same  
 chromophore, **phycocyanobilin**. When two genes in the phycocyanin  
 operon of this organism, *cpcE* and *cpcF*, are inactivated by insertion,  
 together or sep., the surprising result is elimination of correct bilin  
 attachment at only one site, that on the .alpha. subunit of phycocyanin.  
*CpcE* and *CpcF* were overproduced in *Escherichia coli*. In vitro, these  
 proteins catalyze the attachment of **phycocyanobilin** to the  
 .alpha. subunit of apophycocyanin at the appropriate site, .alpha.-Cys-84,  
 to form the correct adduct. *CpE* and *CpcF* also efficiently catalyze the  
 reverse reaction, in which the bilin from **holo**-.alpha. subunit  
 is transferred either to the apo-.alpha. subunit of the same C-phycocyanin  
 or to the apo-.alpha. subunit of a heterologous C-phycocyanin. The  
 forward and reverse reactions each require both *CpcE* and *CpcF* and are  
 specific for the .alpha.-Cys-84 position. **Phycocyanobilin** is  
 the immediate precursor of the protein-bound bilin.  
 ST *Synechococcus* phycocyanin subunit **phycocyanobilin** lyase; gene  
*cpcE cpcF* phycocyanin **phycocyanobilin** lyase  
 IT *Synechococcus*  
 (**phycocyanin .alpha.-subunit  
 phycocyanobilin lyase** of, genes *cpcE* and *cpcF*  
 encoding and specificity of)  
 IT **Phycocyanins**  
 RL: BIOL (Biological study)  
 (**phycocyanobilin** attachment in, of *Synechococcus*, site of and  
 enzyme specific for)  
 IT Gene, microbial  
 RL: BIOL (Biological study)  
 (*cpcF*, **phycocyanin .alpha.-subunit  
 phycocyanobilin lyase** component encoded by, of  
*Synechococcus*)  
 IT Gene, microbial  
 RL: BIOL (Biological study)  
 (*cpcE*, **phycocyanin .alpha.-subunit  
 phycocyanobilin lyase** component encoded by, of  
*Synechococcus*)  
 IT 144378-42-7, **Phycocyanin .alpha.-  
 subunit phycocyanobilin lyase**  
 RL: BIOL (Biological study)  
 (genes *cpcE*- and *cpcF*-encoded, of *Synechococcus*, attachment site  
 specificity of)  
 IT 52-90-4, Cysteine, biological studies  
 RL: BIOL (Biological study)  
 (of phycocyanin .alpha. subunit position 84, of *Synechococcus*, as  
**phycocyanobilin** attachment site)  
 IT 20298-86-6, **Phycocyanobilin**

RL: BIOL (Biological study)  
(of phycocyanin, attachment of, by enzyme of Synechococcus,  
localization of binding site in)

L79 ANSWER 13 OF 14 HCAPLUS COPYRIGHT 2003 ACS

AN 1992:210724 HCAPLUS

DN 116:210724

TI Detection of analytes using fluorescent energy transfer

IN Tsien, Roger Y.; Taylor, Susan S.; Adams, Stephen R.; Ji, Ying

PA University of California, Oakland, USA

SO PCT Int. Appl., 38 pp.

CODEN: PIXXD2

DT Patent

LA English

IC ICM C12Q001-68

ICS G01N033-566; G01N033-533

CC 9-5 (Biochemical Methods)

Section cross-reference(s): 80

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9200388	A1	19920109	WO 1991-US4676	19910701
	W: JP				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
	EP 537270	A1	19930421	EP 1991-913255	19910701
	EP 537270	B1	19980909		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	JP 05508772	T2	19931209	JP 1991-512652	19910701
	JP 3208486	B2	20010910		
	AT 170980	E	19980915	AT 1991-913255	19910701
	US 5439797	A	19950808	US 1993-114103	19930830
PRAI	US 1990-547990	A	19900702		
	WO 1991-US4676	W	19910701		

AB Analytes such as cAMP, GTP, hormone-receptor complexes, Ca<sup>2+</sup>, diacylglycerol, and phorbol esters are detd. by a method involving radiationless energy transfer between 2 fluorochromes, each bound to a protein; the proteins are reversibly assocd. with one another, the equil. between assocd. and dissocd. states being dependent on the analyte concn. Thus, the catalytic subunit of cAMP-dependent protein kinase (I) was labeled with FITC and the regulatory subunit of I with tetramethylrhodamine isothiocyanate, and the 2 subunits were allowed to assoc. to form **holoenzyme**. CAMP was detd. in single smooth muscle cells by microinjection of the cells with doubly labeled **holo-I**, illumination of the cells at 490 nm, and measurement of the ratio of emitted light intensity at 500-530 nm (fluorescein emission) and 580 nm (tetramethylrhodamine emission). The ratio rapidly increased after microinjection of isoproterenol (.beta.2-adrenergic agonist which raises cAMP concn.) or forskolin (adenylate cyclase activator) and decreased by propranolol (.beta.2-adrenergic antagonist). Expression of the genes for the catalytic and regulatory subunits of I in Escherichia coli, purifn. of the recombinant subunits, and purifn. of the catalytic subunit of I from porcine heart for use in the cAMP assay are described.

ST fluorescent energy transfer biochem analysis; protein fluorochrome radiationless energy transfer; cAMP detn fluorometry energy transfer

IT Plasmid and Episome

(62C12, gene for cAMP-dependent protein kinase regulatory subunit on, cloning and expression of, in Escherichia coli)

IT Heart, composition

(cAMP-dependent protein kinase catalytic subunit of, purifn. of)

IT Fluorescent substances

(conjugates with proteins, in fluorometric biochem. anal., radiationless energy transfer between fluorochromes in relation to)

IT Gene, animal

- RL: ANST (Analytical study)  
(for cAMP-dependent protein kinase catalytic and regulatory subunits,  
cloning and expression of, in Escherichia coli)
- IT Receptors  
RL: ANST (Analytical study)  
(for hormones, detn. of, fluorometric, radiationless energy transfer  
between protein-bound fluorochromes in)
- IT Calmodulins  
RL: ANST (Analytical study)  
(in fluorometric biochem. anal., radiationless energy transfer between  
fluorochromes in relation to)
- IT **Molecular cloning**  
(of cAMP-dependent protein kinase catalytic and regulatory subunit  
genes, in Escherichia coli)
- IT Plasmid and Episome  
(pLWS-3, gene for cAMP-dependent protein kinase catalytic subunit on,  
cloning and expression of, in Escherichia coli)
- IT Hormones  
RL: ANST (Analytical study)  
(receptors for, detn. of, fluorometric, radiationless energy transfer  
between protein-bound fluorochromes in)
- IT Proteins, specific or class  
RL: ANST (Analytical study)  
(GTP-binding, fluorochrome-labeled subunits of, in fluorometric  
biochem. anal., radiationless energy transfer between fluorochromes in  
relation to)
- IT Enzymes  
Proteins, specific or class  
RL: ANST (Analytical study)  
(conjugates, with fluorochromes, in fluorometric biochem. anal.,  
radiationless energy transfer between fluorochromes in relation to)
- IT **Allophycocyanins**  
**Phycoerythrins**  
RL: ANST (Analytical study)  
(conjugates, with proteins, in fluorometric biochem. anal.,  
radiationless energy transfer between fluorochromes in relation to)
- IT Glycerides, analysis  
RL: ANT (Analyte); ANST (Analytical study)  
(di-, detn. of, fluorometric, radiationless energy transfer between  
protein-bound fluorochromes in)
- IT Spectrochemical analysis  
(fluorometric, fluorescent-labeled proteins in, radiationless energy  
transfer between fluorochromes in relation to)
- IT Muscle, composition  
(smooth, cAMP detn. in, fluorescent-labeled cAMP-dependent protein  
kinase catalytic and regulatory subunits in, radiationless energy  
transfer between fluorochromes in relation to)
- IT 27072-45-3, FITC  
RL: ANST (Analytical study)  
(cAMP-dependent protein kinase catalytic subunit conjugation with)
- 
- IT 107347-53-5, Tetramethylrhodamine isothiocyanate  
RL: ANST (Analytical study)  
(cAMP-dependent protein kinase regulatory subunit conjugation with)
- IT 60-92-4, CAMP 86-01-1, GTP 7440-70-2, Calcium, analysis 17673-25-5D,  
Phorbol, esters  
RL: ANT (Analyte); ANST (Analytical study)  
(detn. of, fluorometric, radiationless energy transfer between  
protein-bound fluorochromes in)
- IT 2321-07-5D, Fluorescein, protein conjugates 2768-89-0D, Rhodamine X,  
protein conjugates 9026-43-1D, fluorochrome conjugates 19063-57-1D,  
7-Aminocoumarin, derivs., protein conjugates 70281-37-7,  
Tetramethylrhodamine 127409-15-8D, derivs., protein conjugates  
141229-14-3D, protein conjugates

- RL: ANST (Analytical study)  
(in fluorometric biochem. anal., radiationless energy transfer between  
fluorochromes in relation to)
- IT 142008-29-5P  
RL: PREP (Preparation)  
(purifn. of recombinant catalytic and regulatory subunits of, from  
Escherichia coli)
- L79 ANSWER 14 OF 14 HCAPLUS COPYRIGHT 2003 ACS  
AN 1992:52545 HCAPLUS  
DN 116:52545  
TI Expression and assembly of spectrally active recombinant  
**holophytochrome**  
AU Wahleithner, Jill A.; Li, Liming; Lagarias, J. Clark  
CS Dep. Biochem. Biophys., Univ. California, Davis, CA, 95616, USA  
SO Proceedings of the National Academy of Sciences of the United States of  
America (1991), 88(23), 10387-91  
CODEN: PNASA6; ISSN: 0027-8424  
DT Journal  
LA English  
CC 3-2 (Biochemical Genetics)  
Section cross-reference(s): 11  
AB To develop an in vitro phytochrome assembly system, the authors expressed  
an oat phytochrome cDNA in both the yeast *Saccharomyces cerevisiae* and the  
bacterium *Escherichia coli*. Anal. of sol. protein exts. showed that the  
recombinant apophytochromes were full-length and capable of covalently  
attaching the phytochrome chromophore analog **phycocyanobilin**.  
Difference spectra indicated that in vitro-assembled  
**holophytochrome** species were photoreversible; however, max. and  
min. difference absorption values were blue-shifted relative to those of  
the native photoreceptor. Exts. contg. the recombinant apophytochromes  
were also incubated with phytychromobilin, the natural chromophore  
synthesized from biliverdin by cucumber etioplast preps. In these  
expts., the difference spectrum obtained was identical to that of native  
oat **holophytochrome**. These results suggest that the recombinant  
apophytochromes adopt a structure similar to that of the apoprotein  
biosynthesized in vivo. ELISAs were used to quantitate phytochrome  
expression levels in both yeast and *E. coli* exts. These measurements show  
that 62-75% of the phytochrome apoprotein in the sol. protein ext. was  
competent to assemble with bilins to form spectrally active  
**holophytochrome**.  
ST oat recombinant spectrally active **holophytochrome** assembly;  
*Saccharomyces* cloning recombinant oat phytochrome gene; *Escherichia*  
cloning recombinant oat phytochrome gene; **phycocyanobilin**  
phytychromobilin assembly oat recombinant apophytochrome; phytochrome  
**holo** expression assembly oat yeast  
IT Bile pigments  
RL: BIOL (Biological study)  
(assembly of recombinant oat phytochrome apoprotein with, after  
expression in *Escherichia coli* and yeast, for generation of spectrally  
active **holophytochrome**)
- 
- IT **Phytochromes**  
RL: BIOL (Biological study)  
(assembly of spectrally active recombinant, of oat, after expression in  
*Escherichia coli* and yeast)
- IT *Escherichia coli*  
(cloning and expression in, of apophytochrome phyA3 gene of oat,  
assembly of spectrally active recombinant **holophytochrome**  
subsequent to)
- IT *Saccharomyces cerevisiae*  
(cloning and expression in, of apophytochrome phyA3 gene of oats,  
assembly of spectrally active recombinant **holophytochrome**  
subsequent to)

IT Gene, plant  
RL: BIOL (Biological study)  
(for apophytochrome phyA3, of oat, expression in Escherichia coli and yeast of, assembly of spectrally active **holophytochrome** subsequent to)

IT **Molecular cloning**  
(of oat apophytochrome phyA3 coding region, in Escherichia coli and yeast, assembly of spectrally active **holophytochrome** subsequent to)

IT Plasmid and Episome  
(pGphyA3, oat apophytochrome phyA3 gene on, expression in Escherichia coli of, assembly of spectrally active recombinant **holophytochrome** subsequent to)

IT Plasmid and Episome  
(pMphyA3, oat apophytochrome phyA3 gene on, expression in yeast of, assembly of spectrally active recombinant **holophytochrome** subsequent to)

IT Oat  
(recombinant **holophytochrome** of, assembly of spectrally active, after expression in Escherichia coli and yeast)

IT **20298-86-6, Phycocyanobilin** 78249-71-5,  
Phytochromobilin  
RL: BIOL (Biological study)  
(assembly of recombinant oat phytochrome apoprotein with, after expression in Escherichia coli and yeast, for generation of spectrally active **holophytochrome**)

=> sel hit rn  
E1 THROUGH E8 ASSIGNED

=> fil reg  
FILE 'REGISTRY' ENTERED AT 13:50:40 ON 27 MAR 2003  
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.  
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.  
COPYRIGHT (C) 2003 American Chemical Society (ACS)

Property values tagged with IC are from the ZIC/VINITI data file provided by InfoChem.

STRUCTURE FILE UPDATES: 26 MAR 2003 HIGHEST RN 500755-46-4  
DICTIONARY FILE UPDATES: 26 MAR 2003 HIGHEST RN 500755-46-4

TSCA INFORMATION NOW CURRENT THROUGH MAY 20, 2002

Please note that search-term pricing does apply when conducting SmartSELECT searches.

Crossover limits have been increased. See HELP CROSSOVER for details.

---

Experimental and calculated property data are now available. See HELP PROPERTIES for more information. See STNote 27, Searching Properties in the CAS Registry File, for complete details:  
<http://www.cas.org/ONLINE/STN/STNOTES/stnotes27.pdf>

=> s e1-e8

1	9059-22-7/BI
	(9059-22-7/RN)
1	347401-12-1/BI
	(347401-12-1/RN)
1	14875-96-8/BI
	(14875-96-8/RN)
1	20298-86-6/BI

(20298-86-6/RN)  
1 168680-20-4/BI  
(168680-20-4/RN)  
1 124861-40-1/BI  
(124861-40-1/RN)  
1 144378-42-7/BI  
(144378-42-7/RN)  
1 93527-36-7/BI  
(93527-36-7/RN)  
L80 8 (9059-22-7/BI OR 347401-12-1/BI OR 14875-96-8/BI OR 20298-86-6/B  
I OR 168680-20-4/BI OR 124861-40-1/BI OR 144378-42-7/BI OR 93527  
-36-7/BI)

=> d ide can tot

L80 ANSWER 1 OF 8 REGISTRY COPYRIGHT 2003 ACS  
RN **347401-12-1** REGISTRY  
CN Oxidoreductase, ferredoxin:3Z-phycocyanobilin (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN 3Z-Phycocyanobilin:ferredoxin oxidoreductase  
CN Ferredoxin:3Z-phycocyanobilin oxidoreductase  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS, USPATFULL

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
7 REFERENCES IN FILE CA (1962 TO DATE)  
7 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 138:181625  
REFERENCE 2: 138:148684  
REFERENCE 3: 137:365572  
REFERENCE 4: 136:180441  
REFERENCE 5: 136:50278  
REFERENCE 6: 136:2664  
REFERENCE 7: 135:73274

L80 ANSWER 2 OF 8 REGISTRY COPYRIGHT 2003 ACS  
RN **168680-20-4** REGISTRY  
CN Synthase, holophytoerythrocyanin .alpha. subunit (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN Phycoerythrobilin lyase  
CN Phycoerythrocyanin .alpha.-subunit lyase  
CN Phycoerythrocyanin lyase  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS, USPATFULL

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
5 REFERENCES IN FILE CA (1962 TO DATE)  
6 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 138:181625  
REFERENCE 2: 138:148684

REFERENCE 3: 135:340760

REFERENCE 4: 132:331225

REFERENCE 5: 123:250826

L80 ANSWER 3 OF 8 REGISTRY COPYRIGHT 2003 ACS

RN **144378-42-7** REGISTRY

CN Synthase, holophycocyanin .alpha. subunit (9CI) (CA INDEX NAME)

OTHER NAMES:

CN Phycocyanin .alpha.-subunit phycocyanobilin lyase

MF Unspecified

CI MAN

SR CA

LC STN Files: BIOSIS, CA, CAPLUS

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*

5 REFERENCES IN FILE CA (1962 TO DATE)

5 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 136:2664

REFERENCE 2: 133:330194

REFERENCE 3: 130:234673

REFERENCE 4: 121:3687

REFERENCE 5: 117:228825

L80 ANSWER 4 OF 8 REGISTRY COPYRIGHT 2003 ACS

RN **124861-40-1** REGISTRY

CN Phycobiliviolin (9CI) (CA INDEX NAME)

MF Unspecified

CI MAN

SR CA

LC STN Files: AGRICOLA, BIOSIS, CA, CAPLUS, MEDLINE, USPATFULL

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*

6 REFERENCES IN FILE CA (1962 TO DATE)

6 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 138:181625

REFERENCE 2: 138:148684

REFERENCE 3: 127:31653

REFERENCE 4: 114:140070

REFERENCE 5: 112:194035

REFERENCE 6: 112:50824

L80 ANSWER 5 OF 8 REGISTRY COPYRIGHT 2003 ACS

RN **93527-36-7** REGISTRY

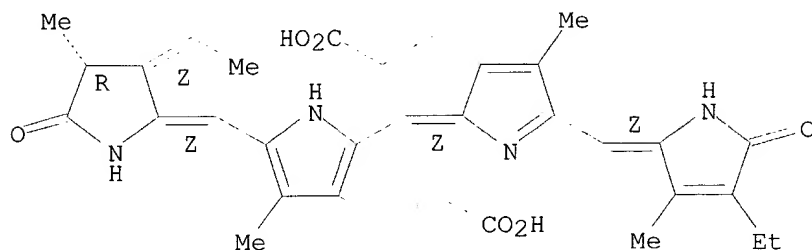
CN 21H-Biline-8,12-dipropionic acid, 18-ethyl-3-ethylidene-1,2,3,19,22,24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxo-, (2R,3Z)- (9CI) (CA INDEX NAME)

OTHER NAMES:

CN 3(Z)-Phycocyanobilin

FS STEREOSEARCH

MF C33 H38 N4 O6

LC STN Files: AGRICOLA, BEILSTEIN\*, BIOSIS, CA, CAPLUS, USPATFULL  
(\*File contains numerically searchable property data)Absolute stereochemistry.  
Double bond geometry as shown.

\*\*PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT\*\*

7 REFERENCES IN FILE CA (1962 TO DATE)  
7 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 138:148684

REFERENCE 2: 136:199818

REFERENCE 3: 136:2664

REFERENCE 4: 127:343697

REFERENCE 5: 116:37656

REFERENCE 6: 115:275429

REFERENCE 7: 102:2991

L80 ANSWER 6 OF 8 REGISTRY COPYRIGHT 2003 ACS

RN 20298-86-6 REGISTRY

CN 21H-Biline-8,12-dipropionic acid, 18-ethyl-3-ethylidene-1,2,3,19,22,24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxo-, (2R,3E)- (9CI) (CA INDEX NAME)

OTHER CA INDEX NAMES:

CN Biline-8,12-dipropionic acid, 18-ethyl-3-ethylidene-1,2,3,19,22,24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxo- (8CI)

CN Phycocyanobilin (6CI, 7CI)

OTHER NAMES:

CN 3(E)-Phycocyanobilin

AR 20714-57-2

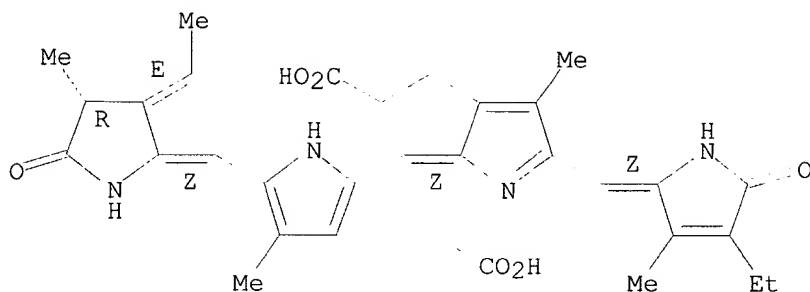
FS STEREOSEARCH

DR 16883-97-9, 18159-29-0, 86747-24-2, 32140-34-4

MF C33 H38 N4 O6

CI COM

LC STN Files: AGRICOLA, BEILSTEIN\*, BIOSIS, CA, CANCERLIT, CAOLD, CAPLUS, DDFU, DRUGU, MEDLINE, TOXCENTER  
(\*File contains numerically searchable property data)Absolute stereochemistry.  
Double bond geometry as shown.



\*\*PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT\*\*

179 REFERENCES IN FILE CA (1962 TO DATE)  
 13 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA  
 180 REFERENCES IN FILE CAPLUS (1962 TO DATE)  
 3 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

REFERENCE 1: 138:181625

REFERENCE 2: 138:132853

REFERENCE 3: 138:68600

REFERENCE 4: 138:21090

REFERENCE 5: 137:275175

REFERENCE 6: 137:229409

REFERENCE 7: 137:151460

REFERENCE 8: 137:121256

REFERENCE 9: 137:89372

REFERENCE 10: 137:44332

L80 ANSWER 7 OF 8 REGISTRY COPYRIGHT 2003 ACS

RN **14875-96-8** REGISTRY

CN Ferrate(2-), [7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-4-2)- (9CI) (CA INDEX NAME)

OTHER CA INDEX NAMES:

CN 21H,23H-Porphine, ferrate(2-) deriv.

CN 21H,23H-Porphine-2,18-dipropanoic acid, 7,12-diethenyl-3,8,13,17-tetramethyl-, iron complex

CN Ferrate(2-), [7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-N21,N22,N23,N24]-, dihydrogen, (SP-4-2)-

CN Protoporphyrin, iron complex (6CI)

OTHER NAMES:

CN 1,3,5,8-Tetramethyl-2,4-divinylporphine-6,7-dipropionic acid ferrous complex

CN Ferroheme

CN Ferroprotoporphyrin IX

CN Hem Fe

CN Heme

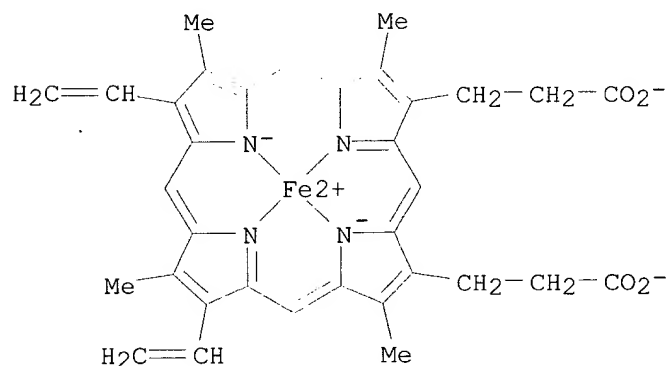
CN Heme b

CN Iron protoporphyrin

CN Iron protoporphyrin IX

CN Iron(II) protoporphyrin IX

CN Protoheme  
 CN Protoheme IX  
 CN Protoporphyrin IX, iron deriv.  
 CN Reduced hematin  
 CN [Dihydrogen 3,7,12,17-tetramethyl-8,13-divinyl-2,18-porphinedipropionato(2-)]iron  
 DR 86-11-3, 69344-57-6, 75197-11-4  
 MF C34 H30 Fe N4 O4 . 2 H  
 CI CCS, COM  
 LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA, CANCERLIT, CAOLD, CAPLUS, CEN, CHEMCATS, CHEMLIST, CIN, DDFU, DRUGU, EMBASE, IFICDB, IFIPAT, IFIUDB, IPA, MEDLINE, MRCK\*, NIOSHTIC, PIRA, PROMT, TOXCENTER, USPATFULL  
 (\*File contains numerically searchable property data)  
 Other Sources: NDSL\*\*, TSCA\*\*  
 (\*\*Enter CHEMLIST File for up-to-date regulatory information)  
 CRN (104414-01-9)



● 2 H<sup>+</sup>

8136 REFERENCES IN FILE CA (1962 TO DATE)  
 364 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA  
 8164 REFERENCES IN FILE CAPLUS (1962 TO DATE)  
 3 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

REFERENCE 1: 138:184613

REFERENCE 2: 138:184252

REFERENCE 3: 138:183658

REFERENCE 4: 138:183001

REFERENCE 5: 138:182997

REFERENCE 6: 138:182996

REFERENCE 7: 138:182995

REFERENCE 8: 138:182991

REFERENCE 9: 138:182973

REFERENCE 10: 138:182788

L80 ANSWER 8 OF 8 REGISTRY COPYRIGHT 2003 ACS  
RN **9059-22-7** REGISTRY  
CN Oxygenase, heme (decyclizing) (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN E.C. 1.14.99.3  
CN Heme oxygenase  
CN ORP33 proteins  
CN Proteins, ORP33 (oxygen-regulated protein, 33,000-mol.-wt.)  
MF Unspecified  
CI MAN  
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO,  
CA, CAPLUS, CEN, CIN, EMBASE, PROMT, TOXCENTER, USPATFULL

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
2206 REFERENCES IN FILE CA (1962 TO DATE)  
23 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA  
2214 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 138:186231  
REFERENCE 2: 138:185239  
REFERENCE 3: 138:185006  
REFERENCE 4: 138:184957  
REFERENCE 5: 138:183158  
REFERENCE 6: 138:182973  
REFERENCE 7: 138:181625  
REFERENCE 8: 138:180708  
REFERENCE 9: 138:180623  
REFERENCE 10: 138:180555

=> d ide can tot 181

L81 ANSWER 1 OF 10 REGISTRY COPYRIGHT 2003 ACS  
RN **347401-21-2** REGISTRY  
CN Oxidoreductase, ferredoxin:3Z-phycoerythrobilin (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN Ferredoxin:3Z-phycoerythrobilin oxidoreductase  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS, USPATFULL

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
4 REFERENCES IN FILE CA (1962 TO DATE)  
4 REFERENCES IN FILE CAPLUS (1962 TO DATE)

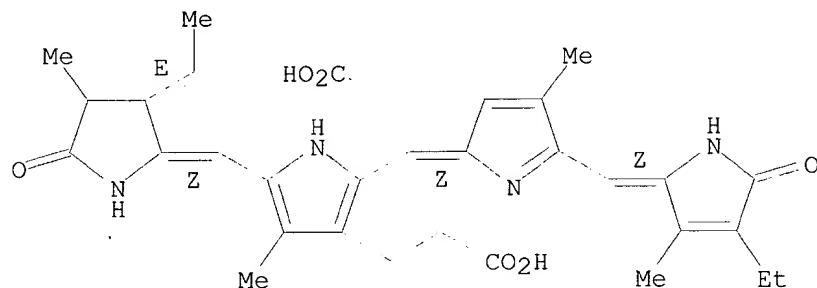
REFERENCE 1: 138:148684  
REFERENCE 2: 137:365572  
REFERENCE 3: 136:50278  
REFERENCE 4: 135:73274

L81 ANSWER 2 OF 10 REGISTRY COPYRIGHT 2003 ACS  
 RN 215871-76-4 REGISTRY  
 CN **21H-Biline-8,12-dipropanoic acid, 18-ethyl-3-ethylidene-1,2,3,19,22,24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxo-, (3E)-(9CI)**  
 (CA INDEX NAME)

## OTHER NAMES:

CN **(.+-.)-Phycocyanobilin**  
 FS STEREOSEARCH  
 DR 322642-34-2  
 MF **C33 H38 N4 O6**  
 SR CA  
 LC STN Files: CA, CAPLUS, CASREACT

Double bond geometry as shown.



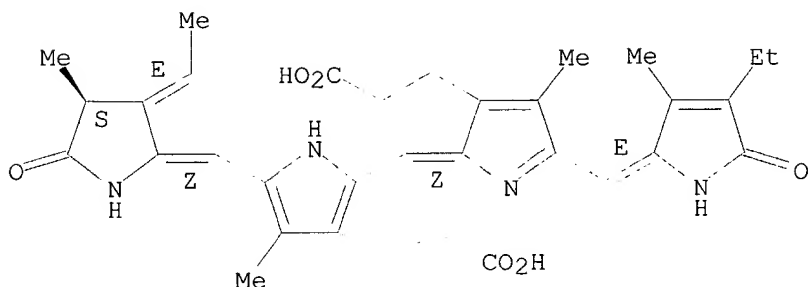
\*\*PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT\*\*

7 REFERENCES IN FILE CA (1962 TO DATE)  
 7 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 135:1857  
 REFERENCE 2: 134:147428  
 REFERENCE 3: 133:120180  
 REFERENCE 4: 132:279038  
 REFERENCE 5: 130:153494  
 REFERENCE 6: 130:3704

L81 ANSWER 3 OF 10 REGISTRY COPYRIGHT 2003 ACS  
 RN 214054-28-1 REGISTRY  
 CN **21H-Biline-8,12-dipropanoic acid, 18-ethyl-3-ethylidene-1,2,3,19,22,24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxo-, (2S,3E,15E)-(9CI)**  
 (CA INDEX NAME)  
 FS STEREOSEARCH  
 MF **C33 H38 N4 O6**  
 SR CA  
 LC STN Files: CA, CAPLUS

Absolute stereochemistry.  
 Double bond geometry as shown.



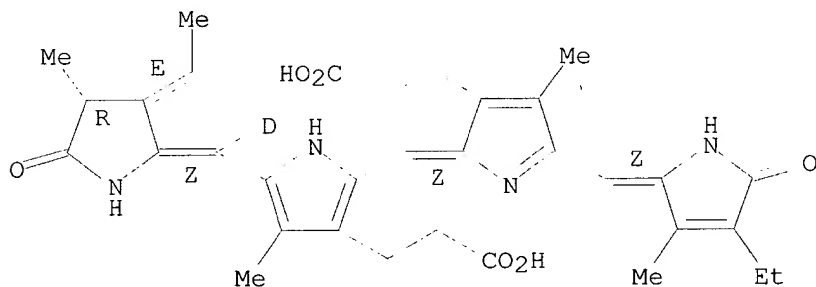
\*\*PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT\*\*

1 REFERENCES IN FILE CA (1962 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 129:289975

L81 ANSWER 4 OF 10 REGISTRY COPYRIGHT 2003 ACS  
RN 189246-94-4 REGISTRY  
CN 21H-Biline-5-d-8,12-dipropanoic acid, 18-ethyl-3-ethylidene-  
1,2,3,19,22,24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxo-, (2R,3E)-  
(9CI) (CA INDEX NAME)  
FS STEREOSEARCH  
MF C33 H37 D N4 O6  
SR CA  
LC STN Files: CA, CAPLUS

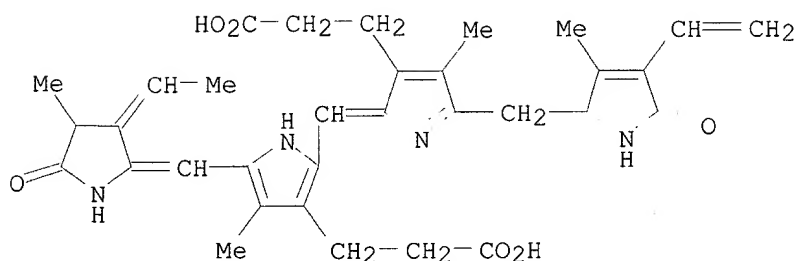
Absolute stereochemistry.  
Double bond geometry as shown.



1 REFERENCES IN FILE CA (1962 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 126:317272

L81 ANSWER 5 OF 10 REGISTRY COPYRIGHT 2003 ACS  
RN 137332-18-4 REGISTRY  
CN 21H-Biline-8,12-dipropanoic acid, 18-ethenyl-3-ethylidene-  
1,2,3,15,16,19,22,24-octahydro-2,7,13,17-tetramethyl-1,19-dioxo-,  
(2R,3Z,16R)- (9CI) (CA INDEX NAME)  
MF C33 H38 N4 O6  
SR CA  
LC STN Files: BEILSTEIN\*, CA, CAPLUS  
(\*File contains numerically searchable property data)



\*\*PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT\*\*

2 REFERENCES IN FILE CA (1962 TO DATE)  
2 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 116:37656

REFERENCE 2: 115:275429

L81 ANSWER 6 OF 10 REGISTRY COPYRIGHT 2003 ACS

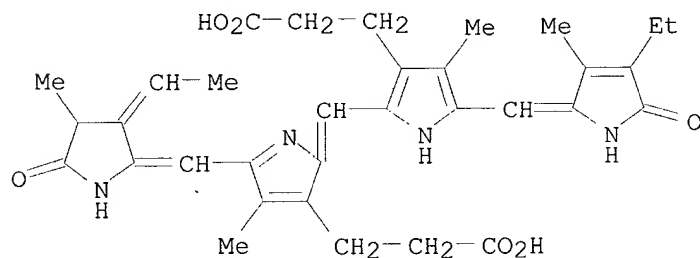
RN 133561-60-1 REGISTRY

CN **21H-Biline-8,12-dipropanoic acid, 18-ethyl-3-ethylidene-1,2,3,19,23,24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxo- (9CI)** (CA INDEX NAME)

MF C33 H38 N4 O6

SR CA

LC STN Files: BEILSTEIN\*, CA, CAPLUS  
(\*File contains numerically searchable property data)



\*\*PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT\*\*

1 REFERENCES IN FILE CA (1962 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 114:201852

L81 ANSWER 7 OF 10 REGISTRY COPYRIGHT 2003 ACS

RN 111565-55-0 REGISTRY

CN Protein LRC27, PC (Nostoc muscorum clone pAn410 27-kilodalton rod-core-linking reduced) (9CI) (CA INDEX NAME)

OTHER NAMES:

CN **Phycocyanobilin lyase alpha subunit (Nostoc sp. PCC 7120 gene cpcE)**

CN Protein (Anabaena PCC7120 strain PCC7120 gene cpcE)

FS PROTEIN SEQUENCE

MF Unspecified

CI MAN

SR CA  
LC STN Files: CA, CAPLUS

**\*\*RELATED SEQUENCES AVAILABLE WITH SEQLINK\*\***

**\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\***  
**\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\***  
3 REFERENCES IN FILE CA (1962 TO DATE)  
3 REFERENCES IN FILE CAPLUS (1962 TO DATE)

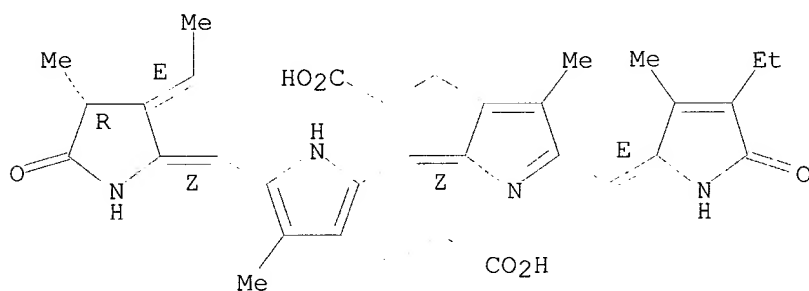
REFERENCE 1: 136:65028

REFERENCE 2: 135:299437

REFERENCE 3: 107:230204

L81 ANSWER 8 OF 10 REGISTRY COPYRIGHT 2003 ACS  
RN 86746-89-6 REGISTRY  
CN **21H-Biline-8,12-dipropanoic acid, 18-ethyl-3-ethylidene-1,2,3,19,22,24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxo-, (2R,3E,15E)-(9CI)** (CA INDEX NAME)  
FS STEREOSEARCH  
MF **C33 H38 N4 O6**  
LC STN Files: BEILSTEIN\*, CA, CAPLUS  
(\*File contains numerically searchable property data)

Absolute stereochemistry.  
Double bond geometry as shown.



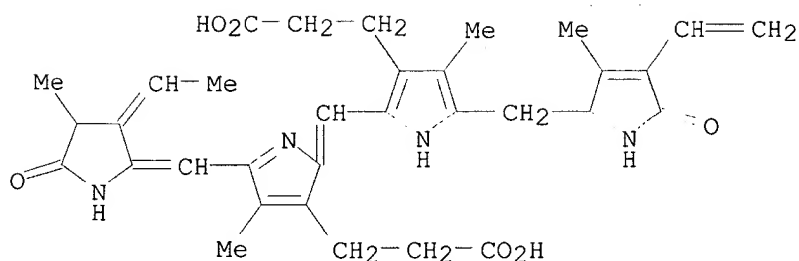
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1 REFERENCES IN FILE CA (1962 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 99:83931

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L81 ANSWER 9 OF 10 REGISTRY COPYRIGHT 2003 ACS  
RN 71189-94-1 REGISTRY  
CN **21H-Biline-8,12-dipropanoic acid, 18-ethenyl-3-ethylidene-1,2,3,15,16,19,23,24-octahydro-2,7,13,17-tetramethyl-1,19-dioxo-, (2R,16R)-(9CI)** (CA INDEX NAME)  
MF **C33 H38 N4 O6**  
LC STN Files: CA, CAPLUS



\*\*PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT\*\*

1 REFERENCES IN FILE CA (1962 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 91:107855

L81 ANSWER 10 OF 10 REGISTRY COPYRIGHT 2003 ACS

RN 18097-67-1 REGISTRY

CN 21H-Biline-8,12-dipropionic acid, 18-ethenyl-3-ethylidene-  
1,2,3,15,16,19,22,24-octahydro-2,7,13,17-tetramethyl-1,19-dioxo-,  
(2R,3E,16R)- (9CI) (CA INDEX NAME)

OTHER CA INDEX NAMES:

CN Biline-8,12-dipropionic acid, 3-ethylidene-1,2,3,15,16,19,22,24-  
octahydro-2,7,13,17-tetramethyl-1,19-dioxo-18-vinyl- (8CI)

OTHER NAMES:

CN 3(E)-Phycoerythrobilin

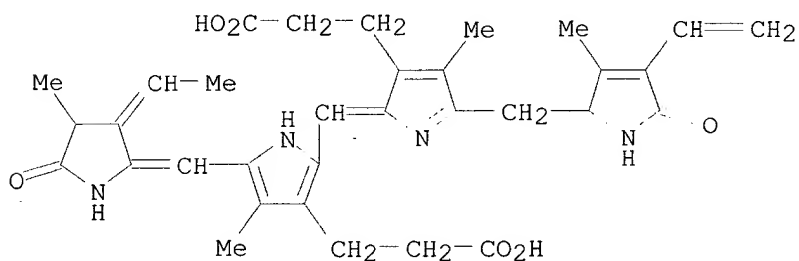
CN Phycoerythrobilin

DR 18159-28-9

MF C33 H38 N4 O6

LC STN Files: AGRICOLA, BEILSTEIN\*, BIOSIS, CA, CAPLUS, MEDLINE, TOXCENTER,  
USPATFULL

(\*File contains numerically searchable property data)



\*\*PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT\*\*

117 REFERENCES IN FILE CA (1962 TO DATE)  
6 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA  
117 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 138:148684

REFERENCE 2: 137:301970

REFERENCE 3: 137:275175

REFERENCE 4: 137:151460

REFERENCE 5: 137:89372  
REFERENCE 6: 136:243489  
REFERENCE 7: 135:354347  
REFERENCE 8: 135:340760  
REFERENCE 9: 135:270216  
REFERENCE 10: 135:73274

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FILE LAST UPDATED: 26 Mar 2003 (20030326/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

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L92 ANSWER 1 OF 5 HCAPLUS COPYRIGHT 2003 ACS

AN 2001:597094 HCAPLUS

DN 135:207297

TI Purification and biochemical properties of phytochromobilin synthase from etiolated oat seedlings

AU McDowell, Michael T.; Lagarias, J. Clark

CS Section of Molecular and Cellular Biology, University of California, Davis, CA, 95616, USA

SO Plant Physiology (2001), 126(4), 1546-1554

---

CODEN: PLPHAY; ISSN: 0032-0889

PB American Society of Plant Physiologists

DT Journal

LA English

CC 7-2 (Enzymes)

AB Plant phytochromes are dependent on the covalent attachment of the linear tetrapyrrole chromophore phytochromobilin (P.PHI.B) for photoactivity. In plants, biliverdin IX.alpha. (BV) is reduced by plastid-localized, ferredoxin (Fd)-dependent phytochromobilin synthase (I) to yield 3Z-P.PHI.B. Here, the >50,000-fold purifn. of I from etioplasts of dark-grown oat (*Avena sativa* L. cv Garry) seedlings is described, using traditional column chromatog. and preparative electrophoresis. Thus, I is a very low abundance enzyme with a robust turnover rate. The turnover rate was estd. to be >100 s<sup>-1</sup>, which is similar to that of mammalian

NAD(P)H-dependent BV reductase. Oat I was a monomer with a subunit mol. wt. of 29 kDa. However, 2 distinct charged forms of I were identified by native isoelec. focusing. The ability of I to reduce BV was dependent on reduced 2Fe-2S Fd. The Km for spinach Fd was detd. to be 3-4 .mu.M. I had a high affinity for its **bilin** substrate, with a submicromolar Km value for BV.

ST phytochromobilin synthase oat seedling

IT Michaelis constant

(of phytochromobilin synthase from etiolated oat seedlings)

IT Oat

(purifn. and characterization of phytochromobilin synthase from etiolated oat seedlings)

IT **138263-99-7P**, Phytochromobilin synthase

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)

(purifn. and characterization of phytochromobilin synthase from etiolated oat seedlings)

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L92 ANSWER 2 OF 5 HCAPLUS COPYRIGHT 2003 ACS

AN 2001:378669 HCAPLUS

DN 135:118588

TI The Arabidopsis HY2 gene encodes phytochromobilin synthase, a ferredoxin-dependent biliverdin reductase

AU Kohchi, Takayuki; Mukougawa, Keiko; Frankenberg, Nicole; Masuda, Munehisa; Yokota, Akiho; Lagarias, J. Clark

CS Graduate School of Biological Sciences, Nara Institute of Science and  
Technology, Nara, 630-0101, Japan

SO Plant Cell (2001), 13(2), 425-436  
CODEN: PLCEEW; ISSN: 1040-4651

PB American Society of Plant Physiologists

DT Journal

LA English

CC 7-2 (Enzymes)

Section cross-reference(s): 3, 11

AB Light perception by the plant photoreceptor phytochrome requires the  
tetrapyrrole chromophore phytychromobilin (P.PHI.B), which is covalently  
attached to a large apoprotein. Arabidopsis mutants hyl and hy2, which  
are defective in P.PHI.B biosynthesis, display altered responses to light  
due to a deficiency in photoactive phytochrome. Here, the authors  
describe the isolation of the HY2 gene by map-based cloning. Hy2 mutant  
alleles possess alterations within this locus, some of which affect the  
expression of the HY2 transcript. HY2 encodes a sol. protein precursor of  
38 kDa with a putative N-terminal plastid transit peptide. The HY2  
transit peptide is sufficient to localize the reporter green fluorescent  
protein to plastids. Purified mature recombinant HY2 protein exhibits  
P.PHI.B synthase activity (i.e., ferredoxin-dependent redn. of biliverdin  
IX.alpha. to P.PHI.B), as confirmed by HPLC and by the ability of the  
**bilin** reaction products to combine with apophytochrome to yield  
photoactive holophytochrome. Database searches and hybridization studies  
suggest that HY2 is a unique gene in the Arabidopsis genome that is  
related to a family of proteins found in oxygenic photosynthetic bacteria.

ST cDNA sequence gene HY2 phytychromobilin synthase Arabidopsis

IT Gene, plant  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
(Biological study)  
(HY2; cDNA and amino acid sequences of gene HY2 phytychromobilin  
synthase of Arabidopsis thaliana)

IT Protein sequences  
cDNA sequences  
(cDNA and amino acid sequences of gene HY2 phytychromobilin synthase of  
Arabidopsis thaliana)

IT Arabidopsis thaliana  
(gene HY2 phytychromobilin synthase of Arabidopsis thaliana and mol.  
characterization of gene HY2)

IT Plastid  
(plastid localization of gene HY2 phytychromobilin synthase of  
Arabidopsis thaliana)

IT 350869-22-6  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
(Biological study)  
(amino acid sequence; cDNA and amino acid sequences of gene HY2  
phytychromobilin synthase of Arabidopsis thaliana)

IT **138263-99-7**, Phytychromobilin synthase  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
(Biological study)  
(gene HY2; gene HY2 phytychromobilin synthase of Arabidopsis thaliana  
and mol. characterization of gene HY2)

IT 328223-94-5, GenBank AB045112  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
(Biological study)  
(nucleotide sequence; cDNA and amino acid sequences of gene HY2  
phytychromobilin synthase of Arabidopsis thaliana)

RE.CNT 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L92 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2003 ACS

AN 2001:312008 HCAPLUS

DN 135:73274

TI Functional genomic analysis of the HY2 family of ferredoxin-dependent  
**bilin** reductases from oxygenic photosynthetic organisms

AU Frankenberg, Nicole; Mukougawa, Keiko; Kohchi, Takayuki; Lagarias, J.  
Clark

CS Section of Molecular and Cellular Biology, University of California at  
Davis, Davis, CA, 95616, USA

SO Plant Cell (2001), 13(4), 965-978

CODEN: PLCEEW; ISSN: 1040-4651

PB American Society of Plant Physiologists

DT Journal

LA English

CC 7-5 (Enzymes)  
 Section cross-reference(s): 10, 11

AB Phytobilins are linear tetrapyrrole precursors of the light-harvesting prosthetic groups of the phytochrome photoreceptors of plants and the **phycobiliprotein** photosynthetic antennae of cyanobacteria, red algae, and cryptomonads. Previous biochem. studies have established that phytobilins are synthesized from **heme** via the intermediacy of biliverdin IX.alpha. (BV), which is reduced subsequently by ferredoxin-dependent **bilin** reductases with different double-bond specificities. By exploiting the sequence of phytochromobilin synthase (HY2) of Arabidopsis, an enzyme that catalyzes the ferredoxin-dependent conversion of BV to the phytochrome chromophore precursor phytochromobilin, genes encoding putative **bilin** reductases were identified in the genomes of various cyanobacteria, oxyphotobacteria, and plants. Phylogenetic analyses resolved four classes of HY2-related genes, one of which encodes red chlorophyll catabolite reductases, which are **bilin** reductases involved in chlorophyll catabolism in plants. To test the catalytic activities of these putative enzymes, representative HY2-related genes from each class were amplified by the polymerase chain reaction and expressed in Escherichia coli. Using a coupled apophytochrome assembly assay and HPLC anal., we examd. the ability of the recombinant proteins to catalyze the ferredoxin-dependent redn. of BV to phytobilins. These investigations defined three new classes of **bilin** reductases with distinct substrate/product specificities that are involved in the biosynthesis of the **phycobiliprotein** chromophore precursors **phycoerythrobilin** and **phycocyanobilin**. Implications of these results are discussed with regard to the pathways of phytobilin biosynthesis and their evolution.

ST **bilin** reductase sequence cyanobacteria plant oxyphotobacteria  
 IT Cyanobacteria  
 Enzyme functional sites  
 Oxyphotobacteria  
 Plant (Embryophyta)  
 Protein sequences  
 (functional genomic anal. of HY2 family of ferredoxin-dependent **bilin** reductases from oxygenic photosynthetic organisms)

IT Evolution  
 (mol.; functional genomic anal. of HY2 family of ferredoxin-dependent **bilin** reductases from oxygenic photosynthetic organisms)

IT Bile pigments  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (phytobilins; functional genomic anal. of HY2 family of ferredoxin-dependent **bilin** reductases from oxygenic photosynthetic organisms)

IT 138263-99-7, Ferredoxin:3Z-phytochromobilin oxidoreductase  
 347401-12-1, Ferredoxin:3Z-**phycocyanobilin** .  
 oxidoreductase 347401-20-1 347401-21-2  
 RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)  
 (functional genomic anal. of HY2 family of **ferredoxin** -dependent **bilin** reductases from oxygenic photosynthetic organisms)

IT 114-25-0, Biliverdin 18097-67-1,  
**Phycoerythrobilin** 20298-86-6, **Phycocyanobilin**  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (functional genomic anal. of HY2 family of ferredoxin-dependent **bilin** reductases from oxygenic photosynthetic organisms)

RE.CNT 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD  
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L92 ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2003 ACS

AN 2000:175309 HCAPLUS

DN 132:204723

TI Purification and characterization of phytochromobilin synthase from Avena sativa

AU McDowell, Michael Thomas

CS Univ. of California, Davis, CA, USA

SO (1999) 147 pp. Avail.: UMI, Order No. DA9940114

From: Diss. Abstr. Int., B 2000, 60(8), 3931

DT Dissertation

LA English

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NEWS	8	Apr 22	Federal Research in Progress (FEDRIP) now available
NEWS	9	Jun 03	New e-mail delivery for search results now available
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NEWS	11	Jun 10	PCTFULL has been reloaded
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NEWS	23	Sep 03	JAPIO has been reloaded and enhanced
NEWS	24	Sep 16	Experimental properties added to the REGISTRY file
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NEWS	27	Oct 21	EVENTLINE has been reloaded
NEWS	28	Oct 24	BEILSTEIN adds new search fields
NEWS	29	Oct 24	Nutraceuticals International (NUTRACEUT) now available on STN
NEWS	30	Oct 25	MEDLINE SDI run of October 8, 2002
NEWS	31	Nov 18	DKILIT has been renamed APOLLIT
NEWS	32	Nov 25	More calculated properties added to REGISTRY
NEWS	33	Dec 02	TIBKAT will be removed from STN
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NEWS	35	Dec 17	PCTFULL now covers WP/PCT Applications from 1978 to date
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NEWS 46 Feb 26 NTIS now allows simultaneous left and right truncation  
 NEWS 47 Feb 26 PCTFULL now contains images  
 NEWS 48 Mar 04 SDI PACKAGE for monthly delivery of multifile SDI results  
 NEWS 49 Mar 19 APOLLIT offering free connect time in April 2003  
 NEWS 50 Mar 20 EVENTLINE will be removed from STN  
 NEWS 51 Mar 24 PATDPAFULL now available on STN  
 NEWS 52 Mar 24 Additional information for trade-named substances without  
 structures available in REGISTRY  
 NEWS 53 Mar 24 Indexing from 1957 to 1966 added to records in CA/CAPLUS  
  
 NEWS EXPRESS January 6 CURRENT WINDOWS VERSION IS V6.01a,  
 CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),  
 AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002  
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=> file medline, biosis, jicst, fsta, wpids, dgene, uspatful		
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=> s c-phycocyanin  
 L1 544 C-PHYCOCYANIN  
  
 => s l1 and holo-alpha subunit

L2 5 L1 AND HOLO-ALPHA SUBUNIT

=> d l2 ti abs ibib tot

L2 ANSWER 1 OF 5 MEDLINE

TI Biosynthesis of a fluorescent cyanobacterial C-  
**phycocyanin holo-alpha subunit** in a  
heterologous host.

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-**phycocyanin** alpha subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in phycobiliprotein biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001504133 MEDLINE

DOCUMENT NUMBER: 21438034 PubMed ID: 11553806

TITLE: Biosynthesis of a fluorescent cyanobacterial C-  
**phycocyanin holo-alpha**  
**subunit** in a heterologous host.

AUTHOR: Toohey A J; Cai Y A; Glazer A N

CORPORATE SOURCE: Department of Molecular and Cell Biology, University of  
California, 142 LSA no. 3200, Berkeley, CA 94720-3200, USA.

SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE  
UNITED STATES OF AMERICA, (2001 Sep 11) 98 (19) 10560-5.  
Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200111

ENTRY DATE: Entered STN: 20010913

Last Updated on STN: 20011105

Entered Medline: 20011101

L2 ANSWER 2 OF 5 MEDLINE

TI Phycocyanin alpha-subunit phycocyanobilin lyase.

AB Phycobiliproteins, unlike other light-harvesting proteins involved in photosynthesis, bear covalently attached chromophores. The bilin chromophores are attached through thioether bonds to cysteine residues. The cyanobacterium *Synechococcus* sp. PCC 7002 has eight distinct bilin attachment sites on seven polypeptides, all of which carry the same chromophore, phycocyanobilin. When two genes in the phycocyanin operon of this organism, cpcE and cpcF, are inactivated by insertion, together or separately, the surprising result is elimination of correct bilin attachment at only one site, that on the alpha subunit of phycocyanin. We have overproduced CpcE and CpcF in *Escherichia coli*. In vitro, these proteins catalyze the attachment of phycocyanobilin to the alpha subunit of apophycocyanin at the appropriate site, alpha-Cys-84, to form the correct adduct. CpcE and CpcF also efficiently catalyze the reverse reaction, in which the bilin from **holo-alpha subunit** is transferred either to the apo-alpha subunit of the same

**C-phyococyanin** or to the apo-alpha subunit of a heterologous **C-phyococyanin**. The forward and reverse reactions each require both CpcE and CpcF and are specific for the alpha-Cys-84 position. Phycocyanobilin is the immediate precursor of the protein-bound bilin.

ACCESSION NUMBER: 92357762 MEDLINE  
DOCUMENT NUMBER: 92357762 PubMed ID: 1495995  
TITLE: Phycocyanin alpha-subunit phycocyanobilin lyase.  
AUTHOR: Fairchild C D; Zhao J; Zhou J; Colson S E; Bryant D A; Glazer A N  
CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, Berkeley 94720.  
CONTRACT NUMBER: GM28994 (NIGMS)  
GM31625 (NIGMS)  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 Aug 1) 89 (15) 7017-21. Journal code: 7505876. ISSN: 0027-8424.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199209  
ENTRY DATE: Entered STN: 19920925  
Last Updated on STN: 19970203  
Entered Medline: 19920904

L2 ANSWER 3 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

TI Biosynthesis of a fluorescent cyanobacterial C-  
**phyococyanin holo-alpha subunit** in a  
heterologous host.

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phyocyanobilin, namely, heme oxygenase 1 and 3Z-phyocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (**C-phyococyanin** alpha subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in phycobiliprotein biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001:482056 BIOSIS

DOCUMENT NUMBER: PREV200100482056

TITLE: Biosynthesis of a fluorescent cyanobacterial C-  
**phyococyanin holo-alpha**  
**subunit** in a heterologous host.

AUTHOR(S): Tooley, Aaron J.; Cai, Yuping A.; Glazer, Alexander N. (1)

CORPORATE SOURCE: (1) Natural Reserve System, University of California  
System, 1111 Franklin Street, 6th Floor, Oakland, CA,  
94607-5200: alexander.glazer@ucop.edu USA

SOURCE: Proceedings of the National Academy of Sciences of the  
United States of America, (September 11, 2001) Vol. 98, No.  
19, pp. 10560-10565. print.  
ISSN: 0027-8424.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

L2 ANSWER 4 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

TI PHYCOCYANIN ALPHA-SUBUNIT PHYCOCYANOBILIN LYASE.

AB Phycobiliproteins, unlike other light-harvesting proteins involved in photosynthesis, bear covalently attached chromophores. The bilin chromophores are attached through thioether bonds to cysteine residues. The cyanobacterium *Synechococcus* sp. PCC 7002 has eight distinct bilin attachment sites on seven polypeptides, all of which carry the same chromophore, phycocyanobilin. When two genes in the phycocyanin operon of this organisms, *cpcE* and *cpcF*, are inactivated by insertion, together or separately, the surprising result is elimination of correct bilin attachment at only one site, that on the .alpha. subunit of phycocyanin. We have overproduced *CpcE* and *CpcF* in *Escherichia coli*. In vitro, these proteins catalyze the attachment of phycocyanobilin to the .alpha. subunit of apophycocyanin at the appropriate site, .alpha. Cys-84, to form the correct adduct. *CpcE* and *CpcF* also efficiently catalyze the reverse reaction, in which the bilin from **holo-.alpha. subunit** is transferred either to the apo-.alpha. subunit of the same **C-phycocyanin** or to the apo-.alpha. subunit of a heterologous **C-phycocyanin**. The forward and reverse reactions each require both *CpcE* and *CpcF* and are specific for the .alpha.-Cys-84 position. Phycocyanobilin is the immediate precursor of the protein-bound bilin.

ACCESSION NUMBER: 1992:506225 BIOSIS

DOCUMENT NUMBER: BA94:124750

TITLE: PHYCOCYANIN ALPHA-SUBUNIT PHYCOCYANOBILIN LYASE.

AUTHOR(S): FAIRCHILD C D; ZHAO J; ZHOU J; COLSON S E; BRYANT D A; GLAZER A N

CORPORATE SOURCE: MCB: STANLEY/DONNER ASU, 229 STANLEY HALL, UNIV. CALIF., BERKELEY, CALIF. 94720.

SOURCE: PROC NATL ACAD SCI U S A, (1992) 89 (15), 7017-7021.  
CODEN: PNASA6. ISSN: 0027-8424.

FILE SEGMENT: BA; OLD

LANGUAGE: English

L2 ANSWER 5 OF 5 USPATFULL

TI Engineering of living cells for the expression of holo-phycobiliprotein-based constructs

AB Recombinant cells which express a fluorescent holo-phycobiliprotein fusion protein and methods of use are described. The cells comprises a bilin, a recombinant bilin reductase, an apo-phycobiliprotein fusion protein precursor of the fusion protein comprising a corresponding apo-phycobiliprotein domain, and a recombinant phycobiliprotein domain-bilin lyase, which components react to form the holo-phycobiliprotein fusion protein. Also described are holo-phycobiliprotein based transcription reporter cells and assays, which cells conditionally express a heterologous-to-the-cell, fluorescent, first holo-phycobiliprotein domain.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2003:37640 USPATFULL

TITLE: Engineering of living cells for the expression of holo-phycobiliprotein-based constructs

INVENTOR(S): Glazer, Alexander N., Berkeley, CA, UNITED STATES  
Tooley, Aaron J., Berkeley, CA, UNITED STATES  
Cai, Yuping, Carmel, IN, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003027285	A1	20030206
APPLICATION INFO.:	US 2001-919486	A1	20010731 (9)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		

LEGAL REPRESENTATIVE: RICHARD ARON OSMAN, SCIENCE AND TECHNOLOGY LAW GROUP,  
75 DENISE DRIVE, HILLSBOROUGH, CA, 94010  
NUMBER OF CLAIMS: 24  
EXEMPLARY CLAIM: 1  
NUMBER OF DRAWINGS: 3 Drawing Page(s)  
LINE COUNT: 918  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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FILE 'MEDLINE, BIOSIS, JICST-EPLUS, FSTA, WPIDS, DGENE, USPATFULL'  
ENTERED AT 16:20:18 ON 26 MAR 2003

L1 544 S C-PHYCOCYANIN  
L2 5 S L1 AND HOLO-ALPHA SUBUNIT

=> s l1 and heterologous host  
L3 2 L1 AND HETEROLOGOUS HOST

=> s l3 and l2  
L4 2 L3 AND L2

=> d l4 ti abs ibib tot

L4 ANSWER 1 OF 2 MEDLINE

TI Biosynthesis of a fluorescent cyanobacterial C-  
**phycocyanin holo-alpha subunit** in a  
**heterologous host.**

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-**phycocyanin** alpha subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in phycobiliprotein biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001504133 MEDLINE

DOCUMENT NUMBER: 21438034 PubMed ID: 11553806

TITLE: Biosynthesis of a fluorescent cyanobacterial C-  
**phycocyanin holo-alpha**  
**subunit in a heterologous host.**

AUTHOR: Tooley A J; Cai Y A; Glazer A N

CORPORATE SOURCE: Department of Molecular and Cell Biology, University of  
California, 142 LSA no. 3200, Berkeley, CA 94720-3200, USA.

SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE  
UNITED STATES OF AMERICA, (2001 Sep 11) 98 (19) 10560-5.  
Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200111  
ENTRY DATE: Entered STN: 20010913  
Last Updated on STN: 20011105  
Entered Medline: 20011101

L4 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

TI Biosynthesis of a fluorescent cyanobacterial C-  
**phycocyanin holo-alpha subunit** in a  
**heterologous host**.

AB The entire pathway for the synthesis of a fluorescent holophycobiliprotein subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-**phycocyanin** alpha subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in phycobiliprotein biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

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DOCUMENT NUMBER: PREV200100482056

TITLE: Biosynthesis of a fluorescent cyanobacterial C-  
**phycocyanin holo-alpha**  
**subunit** in a **heterologous host**.

AUTHOR(S): Tooley, Aaron J.; Cai, Yuping A.; Glazer, Alexander N. (1)

CORPORATE SOURCE: (1) Natural Reserve System, University of California  
System, 1111 Franklin Street, 6th Floor, Oakland, CA,  
94607-5200: alexander.glazer@ucop.edu USA

SOURCE: Proceedings of the National Academy of Sciences of the  
United States of America, (September 11, 2001) Vol. 98, No.  
19, pp. 10560-10565. print.  
ISSN: 0027-8424.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

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NEWS	23	Sep 03	JAPIO has been reloaded and enhanced
NEWS	24	Sep 16	Experimental properties added to the REGISTRY file
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NEWS	27	Oct 21	EVENTLINE has been reloaded
NEWS	28	Oct 24	BEILSTEIN adds new search fields
NEWS	29	Oct 24	Nutraceuticals International (NUTRACEUT) now available on STN
NEWS	30	Oct 25	MEDLINE SDI run of October 8, 2002
NEWS	31	Nov 18	DKILIT has been renamed APOLLIT
NEWS	32	Nov 25	More calculated properties added to REGISTRY
NEWS	33	Dec 02	TIBKAT will be removed from STN
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 NEWS EXPRESS January 6 CURRENT WINDOWS VERSION IS V6.01a,  
 CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),  
 AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002  
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 NEWS INTER General Internet Information  
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 NEWS WWW CAS World Wide Web Site (general information)

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=> s phycobiliproteins  
L1 1893 PHYCOBILIPROTEINS

=> s holophycobiliprotein  
L2 5 HOLOPHYCOBILIPROTEIN

=> d l2 ti abs ibib tot

L2 ANSWER 1 OF 5 MEDLINE  
TI Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo-alpha subunit in a heterologous host.  
AB The entire pathway for the synthesis of a fluorescent **holophycobiliprotein** subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phycocyanin alpha subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in phycobiliprotein biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001504133 MEDLINE  
DOCUMENT NUMBER: 21438034 PubMed ID: 11553806  
TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo-alpha subunit in a heterologous host.  
AUTHOR: Tooley A J; Cai Y A; Glazer A N  
CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, 142 LSA no. 3200, Berkeley, CA 94720-3200, USA.  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Sep 11) 98 (19) 10560-5. Journal code: 7505876. ISSN: 0027-8424.

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PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200111  
ENTRY DATE: Entered STN: 20010913  
Last Updated on STN: 20011105  
Entered Medline: 20011101

L2 ANSWER 2 OF 5 USPATFULL  
TI Engineering of living cells for the expression of holo-phycobiliprotein-based constructs  
AB Recombinant cells which express a fluorescent holo-phycobiliprotein

fusion protein and methods of use are described. The cells comprises a bilin, a recombinant bilin reductase, an apo-phycobiliprotein fusion protein precursor of the fusion protein comprising a corresponding apo-phycobiliprotein domain, and a recombinant phycobiliprotein domain-bilin lyase, which components react to form the holo-phycobiliprotein fusion protein. Also described are holo-phycobiliprotein based transcription reporter cells and assays, which cells conditionally express a heterologous-to-the-cell, fluorescent, first holo-phycobiliprotein domain.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2003:37640 USPATFULL  
 TITLE: Engineering of living cells for the expression of holo-phycobiliprotein-based constructs  
 INVENTOR(S): Glazer, Alexander N., Berkeley, CA, UNITED STATES  
 Tooley, Aaron J., Berkeley, CA, UNITED STATES  
 Cai, Yuping, Carmel, IN, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003027285	A1	20030206
APPLICATION INFO.:	US 2001-919486	A1	20010731 (9)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	RICHARD ARON OSMAN, SCIENCE AND TECHNOLOGY LAW GROUP, 75 DENISE DRIVE, HILLSBOROUGH, CA, 94010		
NUMBER OF CLAIMS:	24		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	3 Drawing Page(s)		
LINE COUNT:	918		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 3 OF 5 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

TI Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo-.alpha. subunit in a heterologous host.

AB The entire pathway for the synthesis of a fluorescent **holophycobiliprotein** subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phycocyanobilin, namely, heme oxygenase 1 and 3Z-phycocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phycocyanin .alpha. subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in phycobiliprotein biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001329835 EMBASE  
 TITLE: Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo-.alpha. subunit in a heterologous host.  
 AUTHOR: Tooley A.J.; Cai Y.A.; Glazer A.N.  
 CORPORATE SOURCE: A.N. Glazer, Natural Reserve System, University of California System, 1111 Franklin Street, Oakland, CA 94607-5200, United States. alexander.glazer@ucop.edu  
 SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (11 Sep 2001) 98/19 (10560-10565).

Refs: 30  
ISSN: 0027-8424 CODEN: PNASA6

COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

L2 ANSWER 4 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
TI Biosynthesis of a fluorescent cyanobacterial C-phyocyanin holo- $\alpha$  subunit in a heterologous host.  
AB The entire pathway for the synthesis of a fluorescent **holophycobiliprotein** subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phyocyanobilin, namely, heme oxygenase 1 and 3Z-phyocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phyocyanin  $\alpha$  subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qualitatively and quantitatively similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin addition took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF. This approach should permit incisive analysis of many remaining questions in phycobiliprotein biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001:482056 BIOSIS  
DOCUMENT NUMBER: PREV200100482056  
TITLE: Biosynthesis of a fluorescent cyanobacterial C-phyocyanin holo- $\alpha$  subunit in a heterologous host.  
AUTHOR(S): Tooley, Aaron J.; Cai, Yuping A.; Glazer, Alexander N. (1)  
CORPORATE SOURCE: (1) Natural Reserve System, University of California System, 1111 Franklin Street, 6th Floor, Oakland, CA, 94607-5200; alexander.glazer@ucop.edu USA  
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (September 11, 2001) Vol. 98, No. 19, pp. 10560-10565. print.  
ISSN: 0027-8424.

DOCUMENT TYPE: Article  
LANGUAGE: English  
SUMMARY LANGUAGE: English

L2 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2003 ACS  
TI Biosynthesis of a fluorescent cyanobacterial C-phyocyanin holo- $\alpha$  subunit in a heterologous host  
AB The entire pathway for the synthesis of a fluorescent **holophycobiliprotein** subunit from a photosynthetic cyanobacterium (*Synechocystis* sp. PCC6803) was reconstituted in *Escherichia coli*. Cyanobacterial genes encoding enzymes required for the conversion of heme to the natural chromophore 3Z-phyocyanobilin, namely, heme oxygenase 1 and 3Z-phyocyanobilin:ferredoxin oxidoreductase, were expressed from a plasmid under control of the hybrid trp-lac (trc) promoter. Genes for the apoprotein (C-phyocyanin  $\alpha$  subunit; cpcA) and the heterodimeric lyase (cpcE and cpcF) that catalyzes chromophore attachment were expressed from the trc promoter on a second plasmid. Upon induction, recombinant *E. coli* used the cellular pool of heme to produce holo-CpcA with spectroscopic properties qual. and quant. similar to those of the same protein produced endogenously in cyanobacteria. About a third of the apo-CpcA was converted to holo-CpcA. No significant bilin addn. took place in a similarly engineered *E. coli* strain that lacks cpcE and cpcF.

This approach should permit incisive anal. of many remaining questions in phycobiliprotein biosynthesis. These studies also demonstrate the feasibility of generating constructs of these proteins in situ for use as fluorescent protein probes in living cells.

ACCESSION NUMBER: 2001:705481 HCAPLUS  
DOCUMENT NUMBER: 136:2664  
TITLE: Biosynthesis of a fluorescent cyanobacterial  
C-phycocyanin holo-.alpha. subunit in a heterologous  
host  
AUTHOR(S): Tooley, Aaron J.; Cai, Yuping A.; Glazer, Alexander N.  
CORPORATE SOURCE: Department of Molecular and Cell Biology, University  
of California, Berkeley, CA, 94720-3200, USA  
SOURCE: Proceedings of the National Academy of Sciences of the  
United States of America (2001), 98(19), 10560-10565  
CODEN: PNASA6; ISSN: 0027-8424  
PUBLISHER: National Academy of Sciences  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 15:45:53 ON 26 MAR 2003)

FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, JICST-EPLUS, FSTA, WPIDS,  
BIOSIS, BIOBUSINESS, JAPIO, HCAPLUS' ENTERED AT 15:48:26 ON 26 MAR 2003

L1 1893 S PHYCOBILIPROTEINS  
L2 5 S HOLOPHYCOBILIPROTEIN

=> s apohycobiliprotein  
L3 0 APOHYCOBILIPROTEIN

=> s fusion protein  
L4 139090 FUSION PROTEIN

=> s heterologous  
L5 184332 HETEROLOGOUS

=> s l5 and l4  
L6 18399 L5 AND L4

=> s l6 and l2  
L7 1 L6 AND L2

=> d l7 ti abs ibib tot

L7 ANSWER 1 OF 1 USPATFULL  
TI Engineering of living cells for the expression of holo-phycobiliprotein-  
based constructs

---

AB Recombinant cells which express a fluorescent holo-phycobiliprotein  
**fusion protein** and methods of use are described. The  
cells comprises a bilin, a recombinant bilin reductase, an  
apo-phycobiliprotein **fusion protein** precursor of the  
**fusion protein** comprising a corresponding  
apo-phycobiliprotein domain, and a recombinant phycobiliprotein  
domain-bilin lyase, which components react to form the  
holo-phycobiliprotein **fusion protein**. Also described  
are holo-phycobiliprotein based transcription reporter cells and assays,  
which cells conditionally express a **heterologous-to-the-cell**,  
fluorescent, first holo-phycobiliprotein domain.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2003:37640 USPATFULL  
 TITLE: Engineering of living cells for the expression of  
 holo-phycobiliprotein-based constructs  
 INVENTOR(S): Glazer, Alexander N., Berkeley, CA, UNITED STATES  
 Tooley, Aaron J., Berkeley, CA, UNITED STATES  
 Cai, Yuping, Carmel, IN, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003027285	A1	20030206
APPLICATION INFO.:	US 2001-919486	A1	20010731 (9)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	RICHARD ARON OSMAN, SCIENCE AND TECHNOLOGY LAW GROUP, 75 DENISE DRIVE, HILLSBOROUGH, CA, 94010		
NUMBER OF CLAIMS:	24		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	3 Drawing Page(s)		
LINE COUNT:	918		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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BIOSIS, BIOBUSINESS, JAPIO, HCAPLUS' ENTERED AT 15:48:26 ON 26 MAR 2003

L1 1893 S PHYCOBILIPROTEINS  
 L2 5 S HOLOPHYCOBILIPROTEIN  
 L3 0 S APOHYCOBILIPROTEIN  
 L4 139090 S FUSION PROTEIN  
 L5 184332 S HETEROLOGOUS  
 L6 18399 S L5 AND L4  
 L7 1 S L6 AND L2

=> s recombinant cell

L8 10678 RECOMBINANT CELL

=> s l8 and protein expression

8 FILES SEARCHED...

L9 2727 L8 AND PROTEIN EXPRESSION

=> s l9 and l2

L10 0 L9 AND L2

=> s l9 and l1

L11 1 L9 AND L1

=> s l11 not l7

L12 1 L11 NOT L7

=> d l11 ti abs ibib tot

L11 ANSWER 1 OF 1 USPATFULL

TI Recombinant phycobiliprotein and phycobiliprotein linker fusion proteins  
and uses therefore

AB This invention is directed to the utilization of the developing methods  
 for molecular manipulation of cyanobacteria and red algae (and  
 potentially cryptomonad algae) to express of **phycobiliproteins**  
 and phycobiliprotein linker fusion proteins and their utilization as  
 phycobiliprotein, phycobilisome and subassembly based reagents. In  
 particular, the present invention relates to a method for a specific  
 binding assay to determine a target moiety which is a member of a

specific binding pair, and provides an improvement in the method comprising using a detectable label which is a fusion protein containing both a phycobiliprotein domain and another domain corresponding to a first member of a specific binding pair, where the fusion protein binds to a second member of the specific binding pair to provide a detectable labeled complex. The domain derived from the first member of the specific binding pair can be directly fused to the phycobiliprotein or phycobiliprotein linker domain or be separated by a spacer that allows correct folding of both domains.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2001:237667 USPATFULL  
 TITLE: Recombinant phycobiliprotein and phycobiliprotein linker fusion proteins and uses therefore  
 INVENTOR(S): Allnutt, F.C. Thomas, Port Deposit, MD, United States  
 Toole, Colleen Mary, New Winson, MD, United States  
 Morseman, John Peter, Columbia, MD, United States

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2001055783	A1	20011227
APPLICATION INFO.:	US 2001-882093	A1	20010618 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-211784P	20000616 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	BROBECK, PHLEGER & HARRISON, LLP, ATTN: INTELLECTUAL PROPERTY DEPARTMENT, 1333 H STREET, N.W. SUITE 800, WASHINGTON, DC, 20005	
NUMBER OF CLAIMS:	46	
EXEMPLARY CLAIM:	1	
LINE COUNT:	1218	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, JICST-EPLUS, FSTA, WPIDS, BIOSIS, BIOBUSINESS, JAPIO, HCAPLUS' ENTERED AT 15:48:26 ON 26 MAR 2003

L1 1893 S PHYCOBILIPROTEINS  
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 L3 0 S APOHYCOBILIPROTEIN  
 L4 139090 S FUSION PROTEIN  
 L5 184332 S HETEROLOGOUS  
 L6 18399 S L5 AND L4  
 L7 1 S L6 AND L2  
 L8 10678 S RECOMBINANT CELL  
 L9 2727 S L8 AND PROTEIN EXPRESSION  
 L10 0 S L9 AND L2  
 L11 1 S L9 AND L1  
 L12 1 S L11 NOT L7

=> s biliprotein

L13 734 BILIPROTEIN

=> s l13 and l8

L14 1 L13 AND L8

=> s l9 and l13

L15 1 L9 AND L13

=> d 115 ti abs ibib tot

L15 ANSWER 1 OF 1 USPATFULL

TI Recombinant phycobiliprotein and phycobiliprotein linker fusion proteins and uses therefore

AB This invention is directed to the utilization of the developing methods for molecular manipulation of cyanobacteria and red algae (and potentially cryptomonad algae) to express of phycobiliproteins and phycobiliprotein linker fusion proteins and their utilization as phycobiliprotein, phycobilisome and subassembly based reagents. In particular, the present invention relates to a method for a specific binding assay to determine a target moiety which is a member of a specific binding pair, and provides an improvement in the method comprising using a detectable label which is a fusion protein containing both a phycobiliprotein domain and another domain corresponding to a first member of a specific binding pair, where the fusion protein binds to a second member of the specific binding pair to provide a detectable labeled complex. The domain derived from the first member of the specific binding pair can be directly fused to the phycobiliprotein or phycobiliprotein linker domain or be separated by a spacer that allows correct folding of both domains.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2001:237667 USPATFULL

TITLE: Recombinant phycobiliprotein and phycobiliprotein linker fusion proteins and uses therefore

INVENTOR(S): Allnutt, F.C. Thomas, Port Deposit, MD, United States  
Toole, Colleen Mary, New Winson, MD, United States  
Morseman, John Peter, Columbia, MD, United States

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2001055783	A1	20011227
APPLICATION INFO.:	US 2001-882093	A1	20010618 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-211784P	20000616 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	BROBECK, PHLEGER & HARRISON, LLP, ATTN: INTELLECTUAL PROPERTY DEPARTMENT, 1333 H STREET, N.W. SUITE 800, WASHINGTON, DC, 20005	

NUMBER OF CLAIMS: 46

EXEMPLARY CLAIM: 1

LINE COUNT: 1218

CAS INDEXING IS AVAILABLE FOR THIS PATENT.